

THE ROLE OF ENTEROPATHOGENIC E. COLI IN INFANTILE DIARRHOEA

Aspects on bacteriology, epidemiology
and therapy

by
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This thesis is based on the following studies referred to by their Roman numerals:

- I Thorén A, Stintzing G, Tufvesson B, Walder M, Habte D. Aetiology and clinical features of severe infantile diarrhoea in Addis Ababa, Ethiopia. *J Trop Pediatr* 1982;28:127-31.
- II Persson BL, Thorén A, Tufvesson B, Walder M. Diarrhoea in Swedish infants. Aetiology and clinical appearance. *Acta Paediatr Scand* 1982; 71:909-13.
- III Thorén A, Cronberg S. Enteropatogena E. coli - en anmälningspliktig sjukdom? *Läkartidningen* 1982;79:3200-3. (English translation)
- IV Thorén A, Wolde-Mariam T, Stintzing G, Wadström T, Habte D. Antibiotics in the treatment of gastroenteritis caused by enteropathogenic Escherichia coli. *J Infect Dis* 1980;141:27-31.
- V Thorén A. Antibiotic sensitivity of enteropathogenic Escherichia coli to mecillinam, trimethoprim-sulfamethoxazole and other antibiotics. *Acta Pathol Microbiol Scand, Sect B* 1980;88:265-8.
- VI Thorén A. Pathogenesis of enteropathogenic Escherichia coli. Studies of enterotoxin production, invasive ability and surface antigens. (Submitted for publication)

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ABBREVIATIONS

CFA	colonization factor antigen
CHO	Chinese hamster ovary
EIEC	enteroinvasive <u>Escherichia coli</u>
ELISA	enzyme-linked immunosorbent assay
EPEC	enteropathogenic <u>Escherichia coli</u>
ESPC	Ethio-Swedish Paediatric Clinic
ETEC	enterotoxigenic <u>Escherichia coli</u>
HA	hemagglutination
HIC	hydrophobic interaction chromatography
LT	heat-labile enterotoxin
MRHA	mannose-resistant hemagglutination
MSHA	mannose-sensitive hemagglutination
ST	heat-stable enterotoxin
VT	Vero cell toxin
WHO	World Health Organization

INTRODUCTION

"During the last five years there has been controversy over the role of EPEC as etiological agents in acute diarrhoea. Recent experiments with an animal model and human challenge studies have demonstrated that EPEC can cause diarrhoea. Little is known about their virulence factors or pathogenic mechanisms. Outbreaks of infantile enteritis associated with EPEC are still reported from tropical countries but it remains difficult to evaluate their significance in sporadic cases. More clinico-epidemiological studies are needed from many geographical areas". (B Rowe (1981), member of the WHO scientific working group of Diarrhoeal Diseases Control Programme).

The problem of infantile diarrhoea

Extent of the problem

Diarrhoeal disease is established as a main killer of children in countries with a poor socioeconomic standard and poor sanitary conditions. In 1975, 500 million episodes of diarrhoea were estimated to occur in children below five years of age with a mortality of 1-4% (94). More than one third of the hospital beds in paediatric wards of these countries are occupied by children with diarrhoea (4). The problem is much aggravated by the vicious interaction with malnutrition disorders. Not only can the episode of diarrhoea make a borderline state of malnutrition overt but the malnutrition disorder itself can perpetuate the diarrhoea, thus obstructing a nutritional rehabilitation (108). Bottle feeding as a substitute for breast-feeding greatly enhances the risk of contracting diarrhoea and malnutrition.

Diarrhoea in developing countries is characterized by an endemic situation aggravated by seasonal epidemic outbreaks. Seasonal epidemic outbreaks, for instance rotavirus disease, are still a problem in modern Europe and North America. Moreover, epidemic outbreaks in institutions such as day nurseries and paediatric wards have been a precarious problem in spite of a general high socioeconomic level (96, 100).

The global impact of infantile diarrhoea has been given appropriate attention and priority by WHO including intensified research programs and public health work (4).

Aetiology of infantile diarrhoea

Miscellaneous organisms as a cause of diarrhoea are extensively reviewed by Bäck, 1979 (14). Among viral causes rotavirus is the primary pathogen of infantile diarrhoea both in endemic and epidemic situations and in all countries (1, 11, 30, 47, 87, 99, 122). Even before the discovery of rotavirus, there were reports strongly indicating a virus agent as a cause of epidemic outbreaks among infants (76).

Among parasitic diseases amoebiasis is still important in developing countries (121). Giardia lamblia has a more global extension and has recently been incriminated as a common cause of nosocomial infections also in industrialized countries like Sweden (18).

Among bacterial agents, Campylobacter fetus has recently been recognized as one of the most important enteropathogens beside established agents such as Shigella, Salmonella and Cholera (1, 11, 9, 10, 47, 111, 127).

Special strains of Escherichia coli have also been established enteropathogens in all age groups including infants. The role of E. coli, especially the so called enteropathogenic serogroups (EPEC) will be the object of this study.

The history of E. coli in diarrhoeal disease

T Escherich (1885) described the bacterium later named after him (33). E. coli was observed in epidemic outbreaks of infantile diarrhoea but also in stools from healthy infants. Lesage (1897) demonstrated that serum from an acute case of an epidemic outbreak agglutinated other E. coli isolates within that epidemic, but not strains from healthy children (77). Serological methods were also successfully employed by Goldschmidt 1933 (51). However,

during the first decades of this century the enteropathogenic so called "dyspepsia-coli" were mainly studied by biochemical methods (1). When Bray 1945 reported the epidemic outbreak of *Bacterium coli neapolitanum* (BCN) it was defined by fermentation reactions (fermentation of sucrose and salicin and slow fermentation of maltose) (11). However, the important result of Bray's studies was the observation that all investigated BCN strains belonged to the same serogroup, later named 0111:B4. Giles et al 1947 and 1948 reported outbreaks of different enteropathogenic strains (48, 49), one of them later proved to be the serogroup 0111:B4, the other 055:B5.

Serotyping of E. coli

The serotyping system developed by Kauffmann, Knipschildt, Vahlne and Ørskov (64, 68, 124, 136) made it hence possible to classify *E. coli* strains isolated during epidemics of diarrhoea. Different lists of strains called enteropathogenic were designed and presented from different institutions. Table 1 shows serogroups considered enteropathogenic by the WHO international *Escherichia* Centre, Copenhagen, according to its director Dr F Ørskov.

It should be underlined that the term enteropathogenic *E. coli* (EPEC) thus is strictly based on epidemiological observations.

Toxin production

The rabbit ileal loop test used by De and Chatterje (1953) to demonstrate the enterotoxin of *Vibrio cholera* (25) was also employed to demonstrate enterotoxins produced by certain strains of *E. coli* (26). One of these toxins was closely related to the cholera toxin and since it is heat-labile it is named *E. coli* LT. LT is now more specifically demonstrated by the Y1 adrenal cell-test, CHO-cell test or ELISA technique (54, 101, 131). LT acts by activating cyclic AMP like the cholera toxin thus causing a net influx of electrolytes and water into the small intestine (43).

Other *E. coli* strains were shown to possess a heat-stable enterotoxin (ST) with or without the presence of LT. ST is demonstrated by the suckling mice

Table 1. Original reports on the enteropathogenicity of *Escherichia coli* O-serogroups. The list includes serogroups considered as enteropathogenic according to F Ørskov, the International Escherichia Centre, Copenhagen.

Serogroup	Epidemiological circumstances	Reference
025	Outbreak of severe infantile diarrhoea in Rostock, GDR 1951.	Ørskov 1954 (134)
026	Severe infantile epidemics in Copenhagen hospitals 1943-1944.	Ørskov 1951 (132)
044	Severe infantile epidemics in Copenhagen hospitals 1943-1944.	Ørskov 1955 (135)
055	Yearlong outbreak in Aberdeen 1947.	Giles et al 1949 (49)
086	Single isolates from infantile diarrhoea cases 1950-1951.	Ørskov 1954 (133)
091	Outbreak in baby care unit, South Germany 1964.	Ruckdeschel & Linzenmeier 1966 (98)
0111	Severe outbreak of summer diarrhoea 1943.	Bray 1945 (11)
0114	Cases of white scours in calves 1951, and hospital outbreak in Birmingham 1952.	Charter 1956 (19)
0119	Sporadic cases of severe infantile diarrhoea in Aberdeen 1952.	Smith 1953 (112)
0125	Nursery outbreak in London 1949.	Taylor & Charter 1952 (117)
0126	Not specified hospitalized case, London 1949.	Taylor & Charter 1952 (117)
0127	Sporadic cases in Mexico City 1951-1952, and epidemic in Philadelphia, Ohio, USA 1953.	Ewing et al 1955 (38)
0128	Sporadic cases of infantile diarrhoea and hospital outbreak in Birmingham, UK 1953.	Taylor & Charter 1955 (118)
0142	Sporadic cases of diarrhoea in Djakarta, Indonesia 1958.	Ørskov et al 1959 (137)
0158	Outbreak in baby care unit, United Kingdom 1972.	Rowe et al 1974 (96)

bioassay (46). ST acts by activating cyclic guanosine-monophosphate (GMP) with the same intestinal response as with LT (44). Both these enterotoxins are plasmid mediated (110). Toxigenic E. coli also possess other plasmids mediating ability to colonize the small intestine (34, 36).

Invasive ability

Some epidemic and sporadic cases with clinical dysentery are caused not by *Shigella* but certain serogroups of E. coli (58, 82). Like *Shigella*, these strains invade the colonic mucosa thus giving rise to a clinical picture sometimes indistinguishable from classical dysentery. In consequence these strains are called entero-invasive. The invasive ability is demonstrated in vitro by the S  r  ny test and HeLa-cell test (29, 109).

Definition of EPEC, ETEC and EIEC

EPEC-strains of "classical" serogroups were rarely demonstrated to be enterotoxigenic or invasive in current test models (50, 52). These findings have put the value of routine serogrouping of E. coli in diarrhoeal disease, especially from sporadic cases, under debate (40, 45, 103). Moreover, enterotoxin-producing and invasive E. coli strains were indeed restricted to a limited number of serogroups but generally not the same as the "classical" ones (97).

Since different types of E. coli have been established as a cause of diarrhoea, a new terminology has been accepted. The name enteropathogenic E. coli (EPEC) is reserved for the classical serogroups. Strains producing LT and/or ST are named enterotoxigenic E. coli (ETEC). Strains with invasive ability are called entero-invasive E. coli (EIEC).

Generally these three groups belong to different serogroups, Table 2. However, it is natural that certain serogroups originally listed among enteropathogenic strains later frequently proved to be enterotoxigenic e.g. 078, or invasive e.g. 0124. The terminology may therefore undoubtedly seem rather confusing.

Table 2. O-serogroups associated to EPEC, ETEC and EIEC.
According to B Rowe 1979 (97)

EPEC	EIEC	ETEC
18, 26*, 44	28ac, 112ac	1, 6*, 7, 8*, 9, 15*
55*, 86, 111*	124, 136, 143	20, 25*, 27*, 60
114, 119*, 125*	144, 152, 164	63*, 75, 78*, 80
126*, 127*, 128*		85, 88, 89, 99
142, 158		101, 109, 114, 115
		128, 148*, 153, 159*

*Commonest

Pathogenesis of EPEC-diarrhoea

In addition to epidemiological observations the virulence of EPEC strains has been demonstrated on adult volunteers. Ferguson and June 1952 induced diarrhoea by feeding healthy adults with an EPEC strain, 0111:B4. Large inoculum doses, 10^6 - 10^{10} bacteria were needed to cause symptoms, but no symptoms developed after feeding volunteers with an E. coli strain (non-EPEC) isolated from a healthy infant (42). These results were confirmed by Levine et al 1978 who fed three different EPEC strains isolated from epidemic infantile outbreaks or control strains to 43 adult volunteers (78).

Searching for enterotoxins

The clinical picture of "cholera infantum" has suggested that EPEC diarrhoea is mediated by enterotoxins. In the case of ETEC, two different enterotoxins have been described, LT and ST, both coded for by plasmids (43, 44, 110).

The rabbit ileal loop test (25), the LT-specific Y1-adrenal cell test (101) and the ST-specific suckling mice assay (46) have been employed on EPEC with varying results (119). As shown by Goldschmidt and DuPont 1976 and Gross et al 1976, classical EPEC strains generally do not produce enterotoxins similar to those of ETEC strains (50, 52).

Konowalchuk 1977 reported the presence of a cytotoxin in some strains of E. coli (70). The cytotoxic (killing) effect was assayed by morphological changes in Vero-cells exposed to a culture filtrate. The morphological changes were different from those mediated by "cyto-tonic" toxins (LT) on Vero-cells. This cytotoxin was abbreviated VT. Scotland et al 1979 employed the Vero cell test on 253 classical EPEC strains belonging to 11 different serogroups (107). None of these strains produced LT or ST. Out of 25 VT positive strains 23 belonged to serogroup 026. Thirty-one strains of serogroup 026 were VT negative. Some observations in this study suggested that the VT-production was coded for by a transmissible plasmid. Unfortunately the VT has not provided a general explanation of toxigenicity in EPEC strains since it seems restricted to few serogroups, mainly 026.

Klipstein et al 1978 used a different, in vitro assay for enterotoxins (67). Ultrafiltrate fractions of E. coli culture supernatants were diluted to different concentrations and perfused through the jejunum of rat. Altered water secretion of the intestine was recorded. EPEC strains (LT-negative, ST-negative, non-invasive) from four different epidemic outbreaks were tested (serogroups 0114:H2, 0127:H6, 0128:H2, 0142:H6). It was shown that filtrate fractions from EPEC strains caused altered water secretion at the same high dilutions as established toxigenic (ETEC) strains. E. coli strains from healthy persons were negative in the assay.

Adherence

Ability of microorganisms to stick to target epithelium is now considered an essential step in most infections (6). Binding to epithelium surfaces may be specifically mediated to cell receptors with so called adhesins (6). During the 1950s Duguid and Brinton (12, 28) studied adhesins of enteropathogenic E. coli and other enterobacteriae. Different kinds of pili or fimbriae could be visualized by electron microscopy.

Bacteria possessing pili structures are often able to agglutinate red blood cells of different animal species. The agglutination could sometimes be inhibited by monosaccharides, especially D-mannose. In this way different patterns of hemagglutination (HA) was found to be associated with different

kinds of pili. An example is Type 1 or common type pili that causes mannose-sensitive HA (MSHA) of erythrocytes from guinea-pig and chicken (28).

By immunization of rabbits with pili-fractions, antisera against presumed colonization factors could be prepared. Evans et al 1975 prepared an antiserum against the ETEC strain H10407 that possessed plasmidborn colonization factor antigen (CFA_I). A mutant of the parent strain that had lost its plasmids (H10407-P) was used to adsorb the antiserum and yield a specific CFA_I antiserum (34). Evans et al also demonstrated that the presence of CFA_I was associated with mannose-resistant HA (MRHA) of human red blood cells (35). Later a second colonization factor (CFA_{II}) was detected in other ETEC strains and this caused MRHA of bovine erythrocytes (36). Accordingly, HA-techniques using erythrocytes from different species has been a screening tool in order to search for adhesive properties of bacteria (63).

The CFA adhesins have hydrophobic properties which can be demonstrated by the so called Hydrophobic Interaction Chromatography (HIC). Bacteria possessing the hydrophobic adhesins bind to Phenyl Sepharose in the presence of buffered ammoniumsulfate (39, 113). The behaviour of classical EPEC strains has not been tested in this assay.

EPEC strains generally lack the CFA adhesins (24). Evans et al 1979 applied HA-techniques in 434 E. coli strains (ETEC, EIEC, EPEC) isolated from diarrhoea cases and 193 E. coli strains from healthy persons (37). The CFA-positive ETEC strains were strongly correlated with a distinct HA-pattern. Although the authors claim that EPEC-strains also have a special (different) HA-pattern, the correlation of EPEC to special HA-reactions appears to be less convincing.

A more direct approach to study colonization of EPEC was successfully employed by Williams et al 1978. An EPEC strain (H19/1) belonging to sero-group 026:H11 was in vitro shown to adhere to fetal intestinal mucosa of human origin (129). The adhesion was associated with colicine production and multiresistance to different antibiotics. All these properties were shown to be caused by transmissible plasmids. Removal of the plasmids using a so called plasmid-curing agent (ethidium-bromide) resulted in a non-adhesive strain (H19/23). Moreover, genetic transfer of plasmids to a recipient

non-adhesive E. coli (E. coli K12, resistant to nalidixic acid) gave an adhesive recipient. Unfortunately these elegant studies have not been reproduced with other EPEC strains.

Cravioto et al 1979 indicated an EPEC specific adhesive factor studying in vitro adhesion to HEp-2 cells. 80% of tested EPEC strains were positive in contrast to CFA-positive ETEC strains or control strains from healthy subjects (24).

Similarities with salmonellosis

Another mode of enteropathogenicity of E. coli was presented by Cantey and Blake 1977. An E. coli strain RDEC-1 of serogroup O15 that causes diarrhoea in rabbits is non-invasive and lacks LT and ST enterotoxin (16). The infection caused by this strain is characterized by the destruction of microvilli and bacterial adherence on luminal surfaces. Cantey and Inman 1981 concluded that the colonization procedure was similar to that of *Salmonella* starting with invasion and multiplication in Peyer's patches later followed by colonization of the luminal surfaces when diarrhoea was present three days after inoculation (17).

Ulshen and Rollo 1980 described a similar histological picture in a seven-week-old girl with chronic diarrhoea (123). The only enteropathogen was E. coli O125:H21 that was isolated from stools and duodenal fluid. The disease subsided only after treatment with gentamicin. Similar observations involving EPEC strains of serogroup O111:B4 and O119:B14 have recently been reported by Clausen and Christie 1982 and Rothbaum et al 1982 (21, 95).

Experimental data of EPEC strains concerning this study (I, II, IV, V) are discussed in part VI.

Epidemiological and clinical features of EPEC-diarrhoea

Infective_dose

Routes of transmission for EPEC-diarrhoea are not quite clear. According to volunteer studies in adults, the required infective dose is high - 10^8 organisms - indicating a food and waterborne disease (42, 78). However, the infective dose for small children has not been established. Moreover, the epidemic spread in institutions and the incidence of positive throat cultures have been suggestive of contact - and even airborne transmission (76). It has also been suggested that EPEC should express a "cyclic" i.e. varying virulence which would influence the infective dose and thus epidemiology (59).

Influence_of_feeding_habits

Early observations demonstrated bottle-fed babies as a high risk group for contracting EPEC-diarrhoea (11, 49, 66). The weaning age is critical when the protective breast-feeding ends and new foods capable of carrying disease are introduced. In developing countries the term "weanling diarrhoea" is established (108). Since breast-feeding tends to be prolonged in developing countries, the age peak of EPEC appears to be delayed to 6-12 months of age. However, reliable reports on age incidence of EPEC in developing countries are few (1, 130).

Age_specificity

In contrast to ETEC and EIEC infections which afflict all age groups, EPEC infections are generally restricted to children under two years of age. Outbreaks in adult populations are rare and commonly associated with food- or waterborne transmission (7, 23, 74, 106, 126).

The reason for this peculiar age specificity is unknown. Neter et al reported 1955 that the presence of hemagglutinating antibodies against serogroups 0111, 055 and 026 was related to age (90). Antibodies were present

in about 50% of sera taken at random from non diarrhoeal children of one year of age. Sero response to EPEC infection is, however, less distinct compared to viral infections for instance, and the question whether EPEC antibodies are protective is not established. It remains to explain why EPEC infection should give a persisting immunity in contrast to other E. coli diarrhoeas.

Socioeconomic conditions. Great Britain as an example

Poor sanitary conditions and malnutrition, that characterize countries with a low socioeconomic standard, are related to a high incidence of endemic diarrhoea. This situation is prevalent in the developing countries today and applies also to the incidence of EPEC. Table 3 refers to some reports on the incidence in developing countries.

The epidemiology of EPEC in Great Britain, reviewed by Rowe 1979, exemplifies the situation in an industrial country (97). Up until the 1930s annual outbreaks of "summer diarrhoea", often associated with a high mortality, occurred in the community - most pronounced in poor and crowded industrial areas. An association with bottle-feeding was also observed. Along with improved general standard and hygiene in the 1940s the summer outbreaks were overshadowed by epidemics affecting infants in hospital and nurseries. These outbreaks predominated during the cold season. Still, bottled-fed babies were at particular risk with mortality rates of over 50%.

During the 1940s and 1950s EPEC became apparent as an important agent in child institutions in all Europe and USA. The list of incriminated sero-groups grew. Since the 1960s, however, the incidence of reported outbreaks has decreased in developed countries although some serious epidemics with high mortality would appear after years of absence (61).

Despite the spectacular and unexplained decreased incidence of EPEC-diarrhoea in industrialized countries, there are recent reports from Canada and Finland describing EPEC as the most common bacterial enteropathogen among small children (55, 86).

Table 3. EPEC incidence in developing countries. (Reference no. within brackets)

Country	EPEC incidence (%)	Most common serogroup	Age group (years)	Number of patients
South Africa	33	?	<2	479
Sudan (Khartoum)	37	0111:B4	<3	150
Ethiopia (Addis Ababa)	6	0111:B4	<14	86
Ethiopia (Addis Ababa)	19	0111:B4	<3	175
Kenya (Nairobi)	8	-	<5	36
India	17	?	<5	206
India	5-18	026:B6	-	-
Indonesia (Djakarta)	20	0127:B8	<2	156
Iran (Rural area)	4	0111:B4	<7	452
(Teheran)	7	0111:B4	<7	650
Malaysia	9	086:B4	<10	3809
Panama (city)	5	055:B5	<2	1819
Colombia	7	055:B5, 0127:B8	<2	1764
Mexico (city)	18	026:B6	<12	50

Design of study	Reference
Summary of 4 controlled studies. Hospitalized patients.	Koornhof et al 1979 (71)
Controlled study. Outpatients.	Erwa et al 1971 (32)
Controlled study. Outpatients.	Stintzing et al 1977 (114)
Controlled study. Rehydration unit.	Thorén et al 1982 (I)
Controlled study. Rehydration unit.	Mutanda 1980 (85)
Controlled study. Hospitalized patients.	Sanyal et al 1977 (104)
Summary of urban and rural surveys.	Sanyal 1981 (105)
Community study.	Lie Kian Joe et al 1966 (79)
Community study. Non diarrhoeal cases included. Patients referred to city hospitals.	Mohadjer & Badalian 1969 (84)
Stool analyses from various parts of the country.	Jegathesan et al 1975 (62)
Controlled study. Outpatients.	Kourany & Vásques 1969 (72)
Uncontrolled. Hospitalized patients.	de Olarte et al 1974 (91)
Controlled study. Hospitalized patients.	Donta et al 1977 (27).

Clinical appearance

Great efforts have been made in order to describe the clinical picture of EPEC-diarrhoea. High mortality rates - around 50% - were reported in many epidemic outbreaks and the disease was most severe with premature and bottle-fed babies (11, 48, 66). Like cholera the severity of infection ranges from symptomless carriers to critically ill patients (66). Recent observations from Ethiopia suggest that EPEC cause a more severe infection in children than ETEC (1, 57).

Among more specific symptoms and signs reported in context with EPEC, the so called "seminal smell" of stools was reported by Bray, 1945 (11) and Kirby et al 1950 (66). This rather odd observation has, however, been abandoned as unreliable. The presence of fever and vomiting is common but not discriminating nor the quality of diarrhoea although it is generally watery. A "toxic" appearance meaning a more deteriorated condition than dehydration alone would explain and a tendency to relapse is reported by several investigators (22, 61, 66, 75).

The incubation time is by Laurell 1952 estimated to 8-12 days never exceeding 20 days or less than 3 days (76). Riley 1971 reports 2-12 days (93). Ferguson and June 1955 demonstrated symptoms during the first 24 hours after feeding adult volunteers with EPEC strains (42).

Treatment of EPEC-diarrhoea

General supportive therapy with fluid and electrolytes should be given all cases of gastroenteritis irrespective of aetiology. Breast-feeding has a protective effect and should be continued during the illness (80). Additional specific treatment with antibiotics has also been used to control epidemic spread of the disease and to treat individual patients. Kirby et al 1950, Laurell 1952 and Valman and Wilmers 1969 reported good experiences of antibiotics in combatting epidemic outbreaks in paediatric wards but this treatment was combined with strict measures of hygiene and isolation technique (66, 76, 125). Nelson 1971 reviewed past experiences of different antibiotic regimens for EPEC-diarrhoea and reported own experiences with

neomycin treatment (89). He concluded that the benefit of antibiotic treatment for the individual course of disease is doubtful due to bacteriological relapses and non-convincing clinical results. He emphasized the scarcity of strictly controlled studies including non-treated control subjects. Mann et al 1969 directly advise against the use of antibiotics in hospital outbreaks (81).

From developing countries many reports consider antibiotics in EPEC infection (5, 91). The investigation by Coetzee and Leary from South Africa 1971 has been extensively quoted, but although they had good experiences of gentamicin in combined oral and parenteral treatment, no untreated controls were included (22).

AIMS OF THE PRESENT INVESTIGATION

to study the present role of EPEC in a developing country (Ethiopia) with a low socioeconomic standard and a high incidence of infantile diarrhoea (I).

to study the present role of EPEC in an industrialized country (Sweden) with a high socioeconomic standard. This includes aspects on EPEC in endemic and epidemic situations (II, III).

to evaluate the role of antibiotics as a complement to established symptomatic therapy (IV) and to indicate possible alternatives in the choice of antibiotics (V).

to search for pathogenicity factors of EPEC using experimental models reported for ETEC strains (VI).

MATERIAL

Patients

Ethiopian children attending the Ethio-Swedish Pediatric Clinic in Addis Ababa were studied for the prevalence of different enteropathogens and clinical appearance as detailed (I). All these patients were admitted to hospital because of dehydration and need of i.v. therapy. Patients without diarrhoea and children attending health service during the study (March through July 1979) served as controls.

Ethiopian children with proven EPEC-diarrhoea, still needing hospital care after initial rehydration, were selected for antibiotic treatment with mecillinam or trimethoprim-sulfamethoxazole. Control patients received only supportive therapy (IV). This study was performed October 1977 through March 1978. Patients with other enteropathogens were excluded by routine bacteriology and microscopy.

Swedish children attending the department of Pediatrics, Malmö General Hospital, November 1978 through July 1979 were studied for the prevalence of different enteropathogens and clinical appearance (II). Children attending health service during the same period served as controls.

A retrospective study of Swedish children reported for EPEC infection was performed using the central register at the epidemiological department, National Bacteriological Laboratory, Stockholm, Sweden (III). Statistical data on epidemiology between 1946 and 1981 were studied. Hospital records on individual patients reported during one year (July 1980 through June 1981) were studied.

Strains

EPEC strains collected in Ethiopia 1977-1979 and Sweden 1978-1979 were used (I, II, IV, V). The strains were transported from Ethiopia on deep agar slopes and stored in Trypticase soy broth containing 15% (wt/vol) glycerol

at -70°C .

The EPEC strain H19/1 (026:K60:H11) and the plasmid-cured derivate H19/23 were kindly supplied by PH Williams and AS Neish, University of Leicester, Leicester, England. The ETEC strain H10407 (078:K80:H11) and the plasmid-cured derivate H10407-P were kindly supplied by DG Evans, Houston, Texas, USA.

An invasive E. coli strain (0124) served as positive control in studies for invasiveness. This strain, E. coli K12 and E. coli ATCC 25922 were kindly supplied by R Möllby, the National Bacteriological Laboratory, Stockholm, Sweden.

METHODS

Clinical investigations

Diarrhoea was defined as a minimum of four not formed stools per day during the 24 hours preceding attendance at the clinic. In study IV, detailed case histories were obtained and physical examination performed by the same investigator (AT), in study I the case history was obtained by a special trained nurse and physical examination by another investigator (GS). Assessment of nutritional status and grading of dehydration was done according to international standard (3, 60).

Handling of stool specimens

Ethiopian stool samples were examined fresh for ova and parasites at the laboratory of ESPC. Stool cultures were taken by rectal swab and transported in Stuart medium modified by Gästrin et al (56). In study IV stool cultures were performed at the laboratory of ESPC. Samples for rotavirus analysis were collected in 2 ml screwcapped glasstubes. All samples were sent by weekly direct flights to Sweden. Samples from Swedish children were obtained in the same way but not examined for parasites.

Identification of EPEC

Five lactose-positive colonies from each stool specimen were subcultured on blood agar. Agglutination was performed on glass slides by polyvalent pool antisera and - if positive - by monovalent antisera. For study IV antisera supplied by Dr H Rische, Wernigerode, GDR were used (IV). For slide agglutination in the other studies and confirmation of O-serotype by tube titration antisera from Wellcome Reagents Ltd, Beckenham, England were used. For tube titration bacterial suspensions were boiled at 100°C for one hour and agglutinated with serial dilutions of antiserum (40). Serogroups contained in the respective antisera are listed in study I and IV. Strains were confirmed as E. coli by the API 20 E system and biochemical reactions to Indole,

Voges-Proskauer (VP), H₂S-formation, motility test, lysine and ornithine.

Other enteropathogens

For details see study I, II, IV. Campylobacter and rotavirus were only looked for in study I and II, ETEC only in study I. Parasites in I and IV.

Enterotoxin assays

All strains were tested for heat-labile enterotoxin (LT) by courtesy of ass prof Roland Möllby (NBL, Stockholm). The Y1 adrenal cell test procedure was used (15). All strains were also tested for heat-stable enterotoxin by the infant mice assay (46). By courtesy of Dr Jack Konowalchuk, Ottawa, Canada, six selected strains were tested by the Vero cell test for the presence of cytotoxins (70).

Assay of invasive capacity

The ability of bacteria to penetrate into a line of tissue culture cells (HeLa-cells) were assayed as described by Pedersen et al (92).

Studies on adhesive properties

For description of hemagglutination procedure, hydrophobic interaction chromatography (HIC) and immunological studies on surface antigens, see part VI.

RESULTS AND DISCUSSION

Patients and controls

It should be underlined that all concerned patients in the investigation were hospitalized. The vast majority of patients were under two years of age with a peak around 12 months (1, 11). Reports concerning outpatients with diarrhoea will give a more varying spectrum of age (30, 114). Three factors should be considered regarding the age of the patients:

1. Relation to aetiology
2. Relation to breast-feeding habits
3. A possible age-correlated general host response to infection

Table 4 relates aetiology to age and breast-feeding habits in hospitalized Ethiopian children. Roughly half of all infections occurred in the weaning period (6-12 months). It is also clear that only a minor part of the children with different aetiology were exclusively breast-fed. Distribution of age and breast-feeding habits were, however, the same regardless of aetiology. The similarities of age distribution concerning different aetiology probably reflect an endemic situation. In contrast, epidemic outbreaks of EPEC infections commonly afflict younger children (61).

The protective role of breast-feeding is also demonstrated in Figure 1, where breast-feeding habits and nutritional status are related to age in patients and controls. The difference between exclusively breast-fed children with diarrhoea and controls is significant below 6 months of age ($p < 0.001$). The content of feeding bottles of Ethiopian children is commonly highly contaminated (116). It seems as if additional feeding with breastmilk during the first six months of life gave an insufficient protection against diarrhoea when the children concomitantly were fed with high doses of dangerous foods.

After the first six months of age the protective role of breast-feeding is no longer observed. Possibly this is explained by an impaired nutritional status in exclusively breast-fed children when breastmilk as a single source of nutrients becomes insufficient for the growing child.

Table 4. Correlation of age and breast-feeding habits to aetiology in Ethiopian children with diarrhoea and positive stool test (n = 166)

Age (months)	Breast-feeding habits	Rotavirus (%)					Campylobacter (%)		ETEC (%)		Shigella (%)		Amoeba & Giardia troph. (%)	
		n = 86	n = 33	n = 22	n = 16	n = 3	n = 6							
0-3		5.8	3.0	9.1	-	-	-							
3-<6		22.1	27.3	18.2	25.0	-	16.7							
6-<12		46.5	48.5	50.0	56.3	33.3	66.7							
12-24		24.4	21.2	22.7	18.8	66.7	16.7							
>24		1.2	-	-	-	-	-							
	Breast-fed only	15.1	18.2	9.1	18.8	-	-							
	Mixed feeding	29.1	36.4	45.5	50.0	-	16.7							
	Formula feeding	55.8	45.5	45.5	31.3	100	83.3							

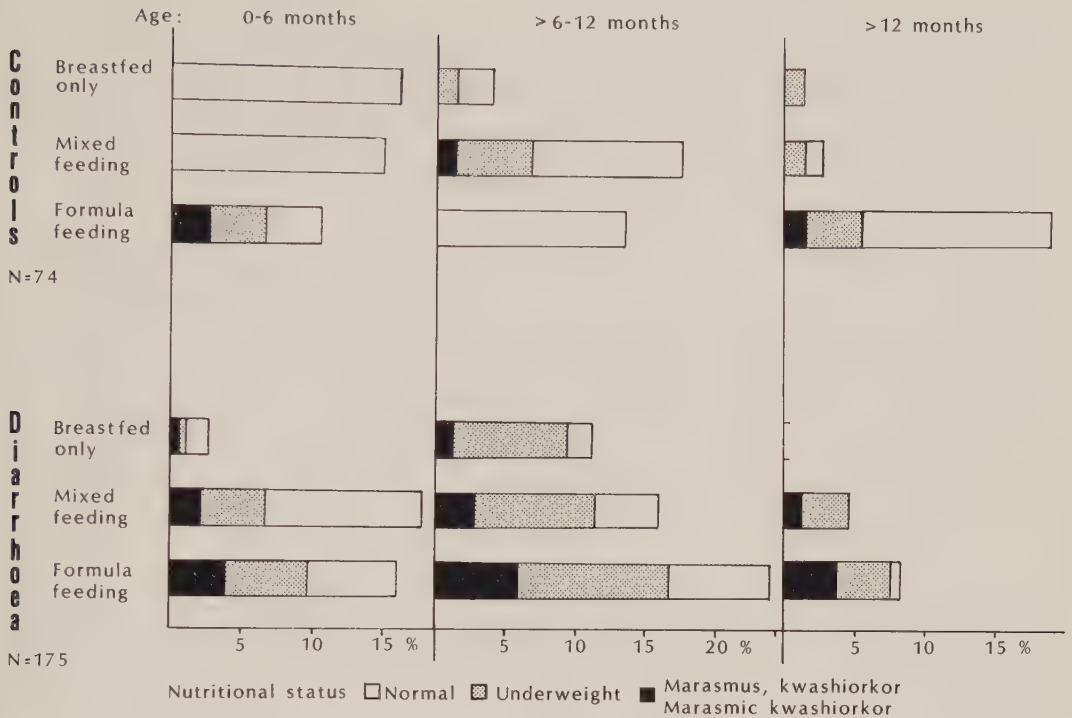


Figure 1. Distribution of age, breast-feeding habits and nutritional status in 175 Ethiopian children with diarrhoea and 74 controls.

Nutritional disorders were not found among Swedish children but frequently among Ethiopian (I, II). In Addis Ababa two groups of protein energy malnutrition (PEM) could be distinguished. The first and most severe consisted of the youngest exclusively formulafed children. This group appeared as victims of the urbanization problems of a developing country. They were often nursed by grandmothers because of split family relations frequently reported from urban centers of poor countries. The second type of PEM was found in older children, over six months, and exclusively breast-fed. The main cause of malnutrition was poverty - the mothers simply could not afford buying foods. Although underweight was common and also - by definition - marasmus, the general appearance of these children was clearly less malignant than in the first group.

Differences in breast-feeding and nutritional status between patients and controls are further discussed in study I. Age- and sexmatched control subjects in study I and II were mostly children coming for vaccination and health check-ups. In Ethiopia some controls were patients with non-diarrhoeal diseases (respiratory infections and surgical cases). The social background of patients versus controls was comparable in both studies.

In summary, the distinct age peak of diarrhoea around 12 months was not related to aetiology. Although exclusive breast-feeding had a protective effect during the first six months of life in Ethiopian children it was not obvious later in life. Since the same peak is observed in Swedish well-nourished and Ethiopian malnourished children it seems as if malnutrition of the weaning period could not alone explain the massive overrepresentation of children below two years of age.

It appears as if the young patient below two years of age responds with more acute symptoms than older children. The need for hospitalization may therefore depend more on age than aetiology.

Microbiological findings

There was a vast difference in occurrence of bacterial diarrhoea when Ethiopian and Swedish children were compared (I, II). Generally this reflects the standard of living. The few cases of bacterial diarrhoea in Swedish children were often of non-domestic origin (II). Carrier state of bacterial pathogens was common in Ethiopian control subjects - 18% versus nil in Sweden (I).

EPEC

In Ethiopia, EPEC was the most frequently isolated bacterial enteropathogen - 19% of all patients (I). No epidemic outbreak occurred during the study and different serogroups were represented, the most common 0111:B4. The findings confirm the role of EPEC in endemic diarrhoea in developing countries. The importance of serogroup 0111:B4 was even more apparent in

study IV where 31 of 49 patients had 0111:B4.

In the Swedish study (II) EPEC was a rare finding (2%) indicating the very limited importance of this enteropathogen in the endemic diarrhoea of industrialized countries.

Other enteropathogens

Rotavirus dominated with 49% of patients in Ethiopia and 58% in Sweden (I, II). In the Swedish study, the typical seasonal winter peak was demonstrated. Stintzing et al could not demonstrate a corresponding clear seasonal variation in Addis Ababa or correlate rotavirus infection to climatology (115).

Among bacterial enteropathogens *Campylobacter* was isolated in 13% of Ethiopian patients and 6% of Swedish (I, II). The hitherto limited experiences of *Campylobacter* epidemiology speak for a worldwide distribution affecting all age-groups (9, 10, 47, 111, 127). Enterotoxigenic bacteria - 9% of Ethiopian children - were nearly as common in controls - 7%. Their role in diarrhoeal disease seems less important in Ethiopia compared to Bangladesh (8). Other enteropathogens (*Shigella*, *Salmonella*, *Yersinia*, *Giardia lamblia* and *Entamoeba histolytica*) were rare findings in Ethiopia. Enterotoxigenic bacteria and parasites were not systematically investigated in study II.

Clinical appearance and symptomatic treatment

The presenting clinical symptoms bringing the child to hospital has been discussed in papers I-III. The correlation of aetiology to presenting symptoms was poor, possibly with exception of dysenteric symptoms which were associated to *Shigella* and sometimes *Campylobacter*.

The forty-nine Ethiopian children with EPEC in study IV represented a selected group. They were all admitted due to EPEC-positive stool cultures and failure to respond to initial intravenous rehydration therapy. However,

it is interesting to observe the long duration of symptoms before admission - 27 patients had diarrhoea more than one week. All patients had persistent watery diarrhoea. Vomiting was observed in 32 patients but in many cases only occasionally. Vomiting is usually most frequent in the acute stage of a gastroenteritis and the dehydrated state of the patients was probably most attributable to profuse watery diarrhoea and/or anorexia. We have also commented on the so called "toxic appearance" of the patients - a lethargic deteriorated general condition that persisted even after adequate rehydration therapy.

Symptomatic treatment of Ethiopian children was always started intravenously as the criterion for admission was the estimated need for intravenous therapy. Forty-one per cent of the patients in study I were rehydrated and discharged within 24 hours, and another 28% within 48 hours. No apparent differences regarding aetiology were observed. In Sweden, criteria for admission were more liberal. A more fortunate access to beds and staff permitted a strictly supervised oral rehydration in 89% of all patients. Hospitalization was extended until complete cure meaning a longer duration (median time 3 days) compared to Ethiopia.

A retrospective survey of notified EPEC cases in Sweden (111) supported the point that during later years EPEC cases were mild and clinically not distinguished from diarrhoeal cases of other aetiology. The serious tendency of epidemic spreads was rarely observed. Moreover, the high mortality rate known from earlier EPEC studies (11, 49) was never reflected in the total infant mortality of diarrhoea; total infant mortality of diarrhoea was compared with the number of notified EPEC cases since 1946. The value of notifying EPEC cases was very limited during the last five years.

Use of antibiotics

Abuse of antibiotics in general and for treatment of diarrhoea in particular has caused great problems with R-factors-borne multiple resistance. Resistant strains of Shigella, Salmonella and EPEC are frequently reported (41, 53, 69).

Unjustified treatment may also impair the condition of the individual patient taking account for side-effects, allergic reactions and disturbed intestinal flora. However, on correct indications, antibiotics may alter the course of disease as shown in study IV. Forty-nine patients with EPEC-infection and failing response to initial rehydration therapy were observed and treated for a minimum of five days. All received appropriate rehydration therapy and 34 received in addition antibiotic therapy (mecillinam or trimethoprim-sulfamethoxazole). The remaining 15 patients served as controls. Complete disappearance of watery stools within three days was taken as clinically successful therapy (see discussion IV). Successful treatment was recorded in 76% of patients with antibiotics versus 7% in controls ($p < 0.001$).

The good response to antibiotic therapy is interesting also from a theoretical point of view. Where do the drugs exert their effect - and accordingly - where is the site of infection? It was observed that although antibiotics made stool cultures EPEC-negative in 53%, this was not correlated with clinical recovery. Five patients included in study IV were also investigated by quantitative culture of duodenal juice before and after antibiotic treatment (116). All five patients had the same EPEC strain in duodenal juice as in stools. After antibiotic treatment, all patients recovered clinically and EPEC disappeared from duodenal juice. Stools became negative in four cases. These findings support earlier observations that the small intestine is the main site of infection (2, 73, 120). More experience is available concerning antibiotics in the treatment of *Shigella* infections (favourable results) and *Salmonella* (doubtful results) (20, 65). The problem of response to antibiotics in enteric infections is complex. Some factors to be considered are listed:

1. Main site of infection: colon - small intestine.
2. Mucosal engagement - intracellular invasion (lymphatic glands).
3. Importance of intraluminal (faecal) persistence.
4. Administrations of drugs: oral - parenteral.
5. Pharmacokinetics of drugs.

It is possible that successful antibiotic treatment of EPEC disease mainly requires adequate serum concentrations of appropriate drugs while faecal concentrations and faecal bacterial persistence is less important.

A varying in vitro susceptibility to different antibiotics as well as the cost and availability of drugs may influence the choice of treatment under different local circumstances. All EPEC strains isolated from patients in study IV were sensitive to mecillinam and trimethoprim-sulfamethoxazole (V). Resistance to four or more antibiotics was shown with 30 strains of 46. Resistance was most commonly observed to ampicillin 65%, chloramphenicol and doxycycline 75% - and sulphonamides 74% of all strains. Antibiotic resistance according to the disc-diffusion method (V) was also assayed in 42 EPEC isolates from study I. Table 5 summarizes the proportion of resistant strains in study I and V.

Table 5. Antibiotic resistance of 88 Ethiopian EPEC isolates to 12 different antibiotics. (Per cent of resistant strains).

MIC ($\mu\text{g/ml}$)	Serogroup 0111:B4 (n=41)	Other serogroups (n=47)
Ampicillin >16	95 %	19 %
Cephalexin >16	0 %	2 %
Gentamicin >16	0 %	0 %
Kanamycin >64	5 %	0 %
Neomycin >128	5 %	0 %
Streptomycin >64	32 %	21 %
Chloramphenicol >8	75 %	36 %
Doxycycline >4	95 %	38 %
Nalidixic acid >32	0 %	0 %
Sulpha-Isodimidin >256	46 %	40 %
Mecillinam >4	0 %	0 %
TMP/SMZ >8	0 %	0 %

Chloramphenicol is widely used in developing countries due to high efficacy and low cost. Transmission of R-factor mediated resistance to many antibiotics and frequent use of chloramphenicol may - in part - explain the common finding of multiresistant strains in Addis Ababa. However, multiresistance of EPEC strains to antibiotics is a common phenomenon with worldwide extension (22, 53, 62, 69, 84, 91, 93). The same is true for Salmonella and Shigella (41). In Addis Ababa, 16 faecal E. coli strains from healthy children were tested for antibiotic susceptibility. All strains were nontoxigenic and had

other serogroups than EPEC. 4/16 strains were resistant to four antibiotics or more. Stintzing et al investigated antibiotic susceptibility to ETEC strains in Addis Ababa and found most strains sensitive (personal communication). The antibiotic susceptibility of ETEC-strains versus other *E. coli* of faecal origin was also reported by Sack et al 1978 (102). However, Echeverria et al 1978 reported contradictory results (31). Ørskov et al 1976 investigated 106 ETEC isolates from different parts of the world (138). Only six strains were resistant to three antibiotics and most strains fully sensitive. It was suggested that plasmids carried by ETEC strains by an incompatibility process hinder the establishment of resistance transfer factors.

The problem of antibiotic multiresistance in EPEC-presumably due to R-factors - impresses the importance of a restrictive policy concerning antibiotic treatment of EPEC infections. It also focuses interest on the possibility of using drugs that do not favour the spread of R-factors. Nalidixic acid represents such a possibility (13), in addition this drug is not required for treating serious systemic manifestations of other diseases and hence problems of resistance to nalidixic acid should be bearable. All EPEC strains investigated in Addis Ababa were fully sensitive to nalidixic acid (V). The clinical use of this drug is somewhat limited by the risk for elevated intracranial pressure in infants younger than four weeks (128) and by economic considerations.

Prophylaxis

EPEC-infections have been associated with a low socio-economic standard and accordingly, they are today most prevalent in countries of the so called third world. The impact of present experiences should guide prophylaxis against:

1. abuse of bottle-feeding to infants
2. poverty and ignorance with its known consequences (crowded households, insufficient and contaminated food, lack of sanitary conveniences).

The development of vaccines against EPEC infections seems more remote. Hitherto no common antigenic factors with pathophysiological significance

are known. Moreover, there appears to be great problems involved in creating effective vaccines against diarrhoeal diseases. The great efforts and priority given to the development of a modern cholera vaccine have not yet responded to previous expectations.

Successful treatment of EPEC diarrhoea has been reported with concentrations of anti-EPEC immunoglobulines given orally (83). Pregnant cows were immunized with anti-EPEC vaccine from different strains. The cow's milk was shown to contain corresponding anti-EPEC immunoglobulines which were concentrated and supplemented to feeding in human babies with EPEC diarrhoea. Theoretically, pregnant women in high-endemic areas could be offered immunization to protect their babies (88).

Epidemic spread of EPEC in institutions must be met with strict isolation techniques. The role of antibiotics is questioned on this indication (81). In study IV, children with EPEC diarrhoea were treated in a general ward without isolation facilities. Although some cases of nosocomial infections occurred there were never any dramatic epidemic spread of EPEC-diarrhoea within the ward.

Pathogenesis of EPEC-diarrhoea

The problems of the pathogenesis of EPEC disease are yet unsolved. The conclusions of a previously presented literature survey and own experimental data (VI) are presented in summary:

1. Epidemiological evidence of virulence has been confirmed by feeding experiments on volunteers (42, 78).
2. The site of infection is the small intestine (2, 21, 73, 95, 116, 120, 123).
3. EPEC strains generally do not possess the pathogenic mechanisms expressed by ETEC and EIEC strains in terms of enterotoxin production (LT/ST), colonization factors (CFA) or invasive capacity (24, 50, 52, VI).

4. Some reports have demonstrated colonization factors (24, 129) and evidence for toxin production (67, 70) in EPEC strains. However, these reports are few and restricted to a limited number of EPEC isolates. A more general model explaining the pathogenesis of EPEC remains to be established.

GENERAL SUMMARY

In endemic circumstances of infantile diarrhoea, the role of enteropathogenic E. coli (EPEC) is important in countries with a general low socio-economic standard. EPEC was the most common bacterial pathogen encountered in the study of 175 Ethiopian children hospitalized for diarrhoea - 19% - compared with an isolation rate of 8% in the 74 control subjects. Serogroup O111:B4 was the most common. The role of EPEC in endemic infantile diarrhoea of children from industrialized countries is nowadays less prominent. In the Swedish study of 95 patients hospitalized for diarrhoea, EPEC was found in only 2% versus none in the 53 control subjects.

Epidemic spread of EPEC disease has also been rare in industrialized countries during the last decades. No distinct outbreaks could be traced during 1975-1980 according to the official Swedish notifying regulations and only one minor institutional outbreak during 1980-1981 could be traced through an intensive retrospective survey. The value of the present Swedish notifying regulations concerning EPEC is doubtful.

Although EPEC infection is commonly associated with severe clinical symptoms, especially in Ethiopia, the clinical picture of EPEC disease is not distinguished from other causes of infantile diarrhoea. Stool analysis is essential to trace epidemic spread of the disease and endemic cases.

Antibiotic therapy proved to be of essential value as a complement to symptomatic therapy in severe clinical cases of EPEC diarrhoea. In the controlled Ethiopian study of 49 patients 76% of antibiotic treated patients fulfilled the postulated criteria of rapid clinical recovery compared with 6% of the controls. The use of antibiotics must be carefully adopted to local circumstances due to the frequent presence of multiresistant strains. In Ethiopia 48/88 strains were resistant to four antibiotics or more.

The pathogenesis of EPEC diarrhoea remains obscure although a colonization of the small intestine and a production of an enterotoxin seem to be important. As in the case of ETEC disease, some pathogenic factors are probably plasmid-

mediated. However, EPEC strains generally do not possess the ETEC associated pathogenic properties (enterotoxin LT/ST, colonization factors CFA). Experimental studies of 78 Ethiopian EPEC isolates failed to demonstrate invasive or ETEC-associated pathogenic properties.

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Articles I - VI

Aetiology and Clinical Features of Severe Infantile Diarrhoea in Addis Ababa, Ethiopia

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Infantile diarrhoea remains one of the leading causes of childhood morbidity and mortality, being most prominent in developing countries. WHO has accordingly underlined the need for epidemiological surveys of infantile diarrhoea in different geographical areas.^{1,2} Children under the age of 2 years are the most commonly affected. The spectrum of recognized intestinal pathogens has increased following recognition of agents such as rotavirus, enterotoxigenic *Escherichia coli* (ETEC) and *Campylobacter fetus ss jejuni*. Several epidemiological investigations have been carried out in developing countries during the last few years in order to establish the role of rotavirus and ETEC.³⁻⁶ Regarding *Campylobacter* infections, however, there are currently only a few reports concerning its role in developing countries.^{7,8} In the City of Addis Ababa infantile diarrhoea is a very serious health problem. In a previous investigation, on patients attending the Outpatients department, we demonstrated the importance and seasonal occurrence of rotavirus and enterotoxigenic bacterial infections in this area.⁴

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The aim of this investigation was to identify the aetiology of diarrhoea in children admitted for intravenous rehydration therapy, and make an attempt to correlate clinical features with aetiology.

Material and Methods

Patients and Controls

Children with diarrhoea ($n = 175$) admitted to the rehydration unit of the Ethio-Swedish Pediatric Clinic, Addis Ababa, Ethiopia (March through June 1979) were included in the study. The study was performed in the beginning of the rainy season which is also the peak season for diarrhoeal diseases.⁴ Children without diarrhoea ($n = 74$) attending the clinic served as controls. The controls were matched for age and sex. Patients treated with broadspectrum antibiotics within one week before admission were excluded. A history, including feeding habits and duration of the presenting symptoms was obtained from the mother by a specially trained nurse. Physical examination was done by one of us (G.S.). This included assessment of nutritional status,⁹ degree of dehydration and signs of concomitant diseases. Rectal temperature and quality of stools were recorded. The quality was graded as watery or not watery, the latter group including loose, mucoid and/or bloody stools. The time required for parenteral hydration therapy was also noted.

Handling of Stool Specimens

Stool specimens were obtained directly on admission and microscopic examination for parasites plus a leucocyte and erythrocyte count was performed on fresh preparations. Only trophozoites of *Giardia lamblia* and *Entamoeba histolytica* were accepted as relevant findings for diarrhoea. Stool samples were also sent on modified Stuart medium¹⁰ to the National Bacteriological Laboratory (NBL) Stockholm, Sweden for enterotoxin bioassay (LT) and to the bacteriological laboratory, General Hospital, Malmö, Sweden for identification of

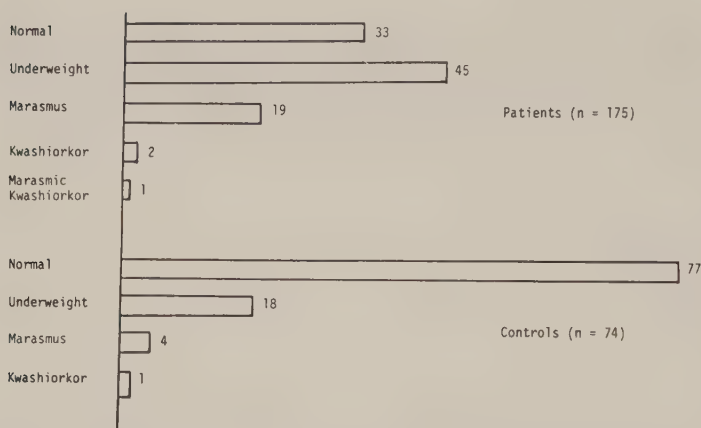


Fig. 1. Nutritional status of patients and controls (%).

Salmonella, *Shigella*, enteropathogenic *E. coli* (EPEC), *Campylobacter fetus ss jejuni*, *Yersinia enterocolitica* and *Vibrios*. All specimens were cultured within 10 days from collection. Stool specimens were also sent to the department of Virology, General Hospital, Malmö, Sweden for rotavirus analysis.

Laboratory Methods

Shigella was cultured on Xylose-Lysine-Desoxycholate agar (XLD-BBL). Enrichment cultures for *Salmonella* were performed in Rappaport tubes for 18 h followed by culture on Lactose-Sacharose-Urea agar, LSU-agar.¹¹ *Yersinia enterocolitica* was cultured on LSU-agar incubated for 18 h in 25°C and also in enrichment broth: 18 h in 5 ml tubes containing one antibiotic disc with carbenicillin (100 µg) and one with cephalothin (30 µg). The broth was then plated on LSU-agar incubated 18 h at 37°C. *Vibrios* were cultured using enrichment procedure for 18 h in alkaline peptone water followed by culture on blood agar and thiosulphate-citrate-bile-salt-sucrose (TCBS) agar. Identification of heat-labile enterotoxin (LT) producing strains was done using the Y1 adrenal cell test as described previously.¹² Only strains giving a positive reaction with >50% of the adrenal cells showing a typical rounded appearance were considered positive.

For isolation of EPEC, 5 lactose positive colonies of *E. coli* were picked from each specimen and subcultured on blood agar after which agglutination was done with polyvalent and monovalent antisera (Wellcome Reagent Ltd, Colindale, England). The antisera contained the following serogroups: 026:B6, 044:K74 (L), 055:B5, 086:B7, 0111:B4, 0114:K90, 0119:B14, 0124:B17, 0125:B15, 0126:B16, 0127:B8, 0128:B12. Strains were confirmed as EPEC by tube titration after heating to 100°C for one hour.¹³ The

EPEC strains were also tested for heat-stable enterotoxin (ST)¹⁴ and invasive properties.¹⁵ Isolation and identification of *Campylobacter fetus ss jejuni* was done according to Skirrow 1977¹⁶. Biochemical identification of all bacterial isolates was performed according to standard methods.¹⁷ Rotavirus was identified by Immune-Electro-Osmo-Phoresis, IEOP.¹⁸

Results

Patients and Controls

Ninetyeight per cent of the patients were under 2 years of age, 0–6 months—37%, 6.5–12 months—51% and 12.5–24 months—10%.

A high number of patients (47%) and controls (43%) were not breastfed. The difference of patients and controls that received only breastmilk up to 6 months of age was significant: 8% of the patients versus 36% of the controls ($p < 0.01$, χ^2 -test). Children with diarrhoea were significantly more malnourished ($p < 0.001$, χ^2 -test), (Fig. 1).

Aetiology

Intestinal pathogens were found in 122 of the 175 patients (70%), compared to 17 of the 74 controls (23%). From one third of the patients more than one, maximum 3 intestinal pathogens were isolated at the time of investigation.

Table 1 shows the isolation rates of different intestinal pathogens. Rotavirus was the most frequent finding encountered in nearly half of the patients. Bacterial pathogens were isolated from 43% of the patients with EPEC as the most frequent finding (19%). Altogether 9 different serogroups were found in patients and controls, representing the following serogroups: 044:K74 (L) 4, 055:B5 9, 086:B7 1, 0111:B4 11, 0114:K90 2, 0119:B14 2,

Table 1
Isolation rates of different enteropathogens

	Patients (n = 175)		Controls (n = 74)		
	No	%	No	%	
Enteropathogenic <i>E. coli</i>	33	19	6	8	$p < 0.05$ (χ^2 -test)
Enterotoxigenic bacteria	15	9	5	7	N.S. (Fischers exact test)
<i>Campylobacter</i>	22	13	2	3	$p < 0.01$ (Fischers exact test)
<i>Shigella</i> spp	3	2	0	0	—
Non Cholera <i>Vibrio</i>	1	1	0	0	—
Rotavirus	85	49	4	5	$p < 0.001$ (χ^2 -test)
<i>G. lamblia</i> *	4	2	1	1	—
<i>E. histolytica</i> *	2	1	0	0	—
No enteropathogens discovered	53	30	57	77	

* Only findings of trophozoites recorded in the table.

0126:B16 4, 0127:B8 6, 0128:B12 1. Two EPEC strains of serogroup 0114:K90 produced heat-labile enterotoxin (LT). All EPEC strains were negative when tested for heat-stable enterotoxin (ST) and invasive properties.

Enterotoxin producing strains (LT) were found in 9% of the patients. Altogether 27 strains were isolated from patients and controls, 22 of these were *E. coli* and 5 represented other enterobacteriaceae (*Citrobacter freundii* 3, *Enterobacter agglomerans* and *Klebsiella pneumoniae*). In one patient 3 different ETEC strains were isolated, while in 2 patients ETEC plus enterotoxigenic non-*E. coli* were isolated. One control case had 2 different ETEC strains, and another an enterotoxigenic *Citrobacter freundii* as the only finding.

No samples were found to contain *Salmonella*, *Yersinia enterocolitica* or *Vibrio cholerae*. One patient had a non-cholera *Vibrio*.

Clinical Symptoms Related to Aetiology

Quality of stools, duration of diarrhoea, rectal temperature and severity of dehydration are related to aetiology in Table 2. Watery diarrhoea and fever was common with EPEC patients while not watery diarrhoea was more common with ETEC patients, although these differences were not significant.

Faecal counts of leucocytes and erythrocytes did not correlate to any specific intestinal pathogen with the exception of the few patients with *Shigella* who all had high leucocyte counts. Neither did age, breast-feeding habits, nutritional status or time needed for parenteral hydration therapy show any correlation to aetiology. The clinical features of diarrhoea were similar in patients having one intestinal pathogen only, compared to patients with double infections.

Five patients died (3%), 4 of them were malnourished and not breastfed. All 5 had rotavirus infection, one also had *Campylobacter* and 2 had EPEC.

Discussion

Severe infantile diarrhoea caused by certain bacterial pathogens such as *Shigella*, EPEC and possibly *Campylobacter* is an indication for antimicrobial therapy^{13,16,19}. In this regard it was interesting to investigate the aetiology of diarrhoea in hospitalized children and relate clinical parameters to aetiology. Concerning the latter aspect, results were discouraging, suggesting that the clinical features of infantile diarrhoea are rather nonspecific when related to aetiology.

The microbiological investigations yielded a high

Table 2
Quality of stools, duration of diarrhoea, fever and dehydration related to aetiology.
(Patients with mixed infections were excluded)

	Watery diarrhoea	Diarrhoea > 14 days	Temperature $\geq 38.0^\circ\text{C}$	Severe dehydration
Enteropathogenic <i>E. coli</i>	9/10	1/10	7/10	3/10
Enterotoxigenic bacteria	2/8	1/8	3/8	4/8
<i>Campylobacter</i>	5/10	1/10	4/10	3/10
Rotavirus	28/47	6/47	16/47	8/47
All diarrhoeal cases	105/175	24/175	67/175	30/175

total isolation rate (70%) compared to many previous studies. Still 30% of the diarrhoeas are aetiologically unexplained. Part of this gap might be filled when other recently discovered viral agents are included in comprehensive studies. The relatively high isolation rate of intestinal pathogens in the control group (23%) underlines the need for control groups in epidemiological studies. The results confirm the important role of rotavirus in this age group. Enterotoxigenic bacteria seem less important particularly since they were also a frequent finding among the controls. In this study the stool specimens were only tested for heat-labile enterotoxin (LT). However, enterotoxigenic strains producing heat-stable enterotoxin (ST) only, seem to be rare in this population (Stintzing *et al.*, unpublished data).

E. coli of classical serogroups (EPEC) was the most frequent bacterial pathogen encountered, as it was in a recent report from South Africa.²⁰ This is interesting since the relevance of EPEC serogrouping in endemic cases of diarrhoea has been under debate.²¹ Also *Campylobacter* infections, of which there are still very few reports from developing countries seems to be an important bacterial pathogen.

In other geographical areas such as Bangladesh, *Vibrio cholerae* is a common bacterial agent.²² Epidemics of cholera have not occurred for many years in Ethiopia (1973) and in this study the only finding was a non-cholera *Vibrio* which probably accounts for some sporadic cases of diarrhoea also in Ethiopia.²³

The vast majority of children were less than 2 years of age. The most affected group, 6–12 months, coincides with the weaning period, and the source of infection is presumed to be contaminated food and water.²⁴ In this aspect early weaning and the increasing incidence of bottle feeding is alarming. The poor nutritional status among patients compared to controls cannot be explained by different breastfeeding habits only. The episode of diarrhoea bringing the child to hospital may have been one of many, with successive impairment of the nutritional status of the child perpetuating the vicious cycle of malnutrition and diarrhoea.

In this comprehensive study, rotavirus, EPEC and *Campylobacter* were the most important aetiological agents. However, to establish the aetiology based on only clinical features seem to be impossible in this area.

The application of new and simple diagnostic techniques in addition to relevant epidemiological information is essential in coping with the problem of infantile diarrhoea in developing countries.² The implications of such obtained results include promotion of breastfeeding, development of the most desirable vaccines, and specified indications for antibiotic therapy.

Summary

This study, performed at Ethio-Swedish Pediatric Clinic, Addis Ababa, Ethiopia, from March through June 1979, included 175 children with diarrhoea in need of parenteral rehydration and 74 age and sex matched controls. Stool samples were analyzed using microscopy, routine bacteriology including culture for *Campylobacter fetus ss jejuni*, enterotoxin bioassay (LT) and rotavirus analysis. The clinical appearance of patients and stools was recorded. A microbiological diagnosis was established in 70% of the patients as compared to 23% positive findings in the controls. The most common findings were rotavirus (49%), enteropathogenic *E. coli* (EPEC) (19%) and *Campylobacter* (13%) of the patients. Among bacterial enteropathogens EPEC was the most prevalent. The isolation rate of enterotoxigenic bacteria was 9% in patients, not differing significantly from the controls, 7%. The correlation of clinical symptoms to aetiology was not conclusive suggesting that the clinical picture of infantile diarrhoea in most cases is nonspecific.

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DIARRHOEA IN SWEDISH INFANTS

Aetiology and Clinical Appearance

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ABSTRACT. Persson, B. L., Thorén, A., Tufvesson, B. and Walder, M. (Departments of Paediatrics, Infectious Diseases, Sections of Clinical Virology and Bacteriology, Department of Medical Microbiology, University of Lund, Malmö General Hospital, Malmö, Sweden). Diarrhoea in Swedish infants. *Acta Paediatr Scand*, 71: 909, 1982.—Ninety-five children with gastroenteritis admitted at the Department of Paediatrics, Malmö General Hospital, Malmö, Sweden, starting November 1978 through October 1979 were studied. Patients were examined clinically and the course of their disease was followed. Stool specimens from all patients were analysed by routine bacteriology and rotavirus identification was done by immune-electrophoresis. Fifty-eight per cent of patients had rotavirus. *Campylobacter* was found in 6 %, enteropathogenic *E. coli* in 2 %, *Yersinia enterocolitica* in 2 % and *Salmonella* in 1 % of patients. Stool specimens from 53 healthy children matched for sex and age served as controls for bacterial pathogens. All were negative. Forty per cent of patients presented with a clinical dehydration, but only 11 %—half of them with bacterial enteropathogens—needed i.v. therapy and 4 % required antibiotic treatment. Median time for hospitalization was three days. Vomiting, fever, watery diarrhoea and initial clinical dehydration were more commonly associated with rota virus infection. Macroscopic blood in stools was restricted to patients with bacterial aetiology.

KEY WORDS: Infants, infectious diarrhoea, rota virus, *Campylobacter jejuni*, enteropathogenic *E. coli*, *Yersinia enterocolitica*

In recent years our knowledge of the aetiology of diarrhoeal diseases in children has expanded with the discovery of new viral and bacterial microorganisms such as rotavirus, enterotoxigenic *E. coli* (ETEC) and *Campylobacter jejuni*. The management of diarrhoeal disease has also focused interest since oral rehydration therapy seems to be effective even in severe cases such as cholera. Accordingly, WHO has called for epidemiological studies of infantile diarrhoea from different parts of the world (1). Recently such studies have been published from Finland (2, 3). The aim of this study was to establish the role of "newer" enteropathogens (rotavirus and *Campylobacter jejuni*) in relation to previously known agents such as for instance enteropathogenic *E. coli* (EPEC) and *Yersinia enterocolitica*.

Further we wished to correlate the clinical signs and symptoms with aetiology.

METHODS

Patients

The study was performed from November 1978 through October 1979 at the Paediatric Clinic, Malmö General Hospital, Malmö, Sweden. During this period, diarrhoea was the main diagnosis in eight per cent of all inpatients. Ninety-five consecutive cases admitted for diarrhoea were included in the study. Patients were admitted to hospital when oral rehydration at home had failed or seemed hazardous due to vomiting and/or dehydration. Fifty-three healthy children, matched for age and sex, attending of the clinic's health service during the same period served as controls for carrier-state of bacterial pathogens. Rotavirus were not investigated among controls. Clinical data were obtained including age, sex, duration of symptoms before hospital contact, appearance of stools (graded as watery or not watery) and the presence of fever, vomiting and concomitant respiratory symp-

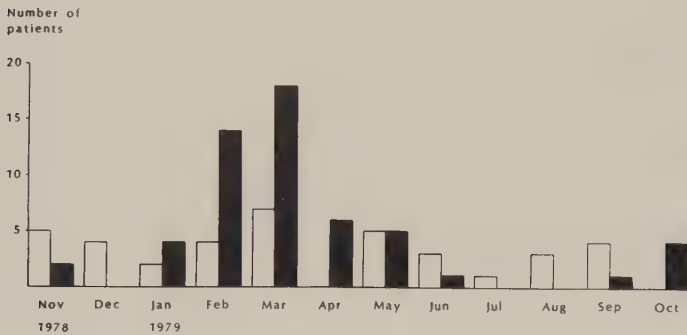


Fig. 1. Monthly distribution of patients with diarrhoea. □, rotavirus-negative patients; ■, rotavirus-positive patients.

toms. Dehydration was graded as mild, moderate or severe (4). The duration of hospital stay as well as intravenous treatment and antibiotic therapy given for the diarrhoeal disease were recorded.

Oral rehydration therapy was carried out as follows: during the first 12 to 24 hours fluid was given as slightly sweetened tea or carrot soup plus table salt (Na^+ about 60 mmol/l). The volume corresponded to 125 ml/kg bodyweight per 24 hours divided in 5–6 meals. This initial nutrition pause was followed by successive introduction of nutrients in a glutenfree diet during a five day period.

The following children were excluded from the study: patients with nosocomial infections, patients on broad-spectrum antibiotics or erythromycin and patients with food intolerance.

The chi-square test was used in the statistical analyses.

Laboratory methods

Stool samples taken as rectal swabs were obtained from all patients and controls. They were cultured by current methods for *Salmonella*, *Shigella*, *Campylobacter jejuni*, *Yersinia enterocolitica* and EPEC (5, 6).

The EPEC antisera were manufactured by the National Bacteriological Laboratory (NBL), Stockholm, Sweden and contained the following O-serogroups: O26, O44, O55, O78, O86, O111, O114, O119, O124, O125, O126, O127, O128. The serogroup O78 has been connected with epidemic infantile diarrhoea (7) but is today more frequently reported as enterotoxin producing strain, ETEC. The EPEC isolates were tested for production of heat labile enterotoxin (LT) performed at NBL, production of heat stable enterotoxin (ST) and invasive capacity (8, 9, 10). For analysis of rotavirus, a 1 ml volume of stools were analysed (11).

RESULTS

Aetiology and source of infections

All cases of diarrhoea were of domestic origin with the exception of three patients returning from the Mediterranean area. Two of them had *Salmonella* and *Campylobacter* respectively while no diagnosis could be established for the third patient.

Isolation rates of intestinal pathogens are shown in Table 1. Rotavirus being the the most important cause of diarrhoea in this study was found in 58% of the cases, all infected in Sweden.

Campylobacter was found in six patients. One of them had a triple infection of *Campylobacter*, rotavirus and EPEC, another *Campylobacter* + rotavirus. Two patients had EPEC infection, the one previously mentioned of serogroup O127: B8, the other one O78. The EPEC strains were negative concerning enterotoxin production (LT + ST) and invasive capacity. Another two patients had *Yersinia enterocolitica* serotype 3. There was one patient with *Salmonella*, but no one with *Shigella*. No bacterial enteropathogens were found among the controls.

Seasonal and age distribution

Monthly distribution of patients with diarrhoea is shown in Fig. 1, the peak rate occurring in the months of February and March.

Table 1. Aetiology of patients with diarrhoea

	Patients (n=95)
Rotavirus	55 (58%) ^{a, b}
<i>Campylobacter</i>	6 (6%) ^{a, b}
EPEC	2 (2%) ^b
<i>Yersinia</i>	2 (2%)
<i>Salmonella</i>	1 (1%)
Unknown aetiology	32 (34%)

^a One patient with Rotavirus and *Campylobacter*.

^b One patient with Rotavirus, *Campylobacter* and EPEC.

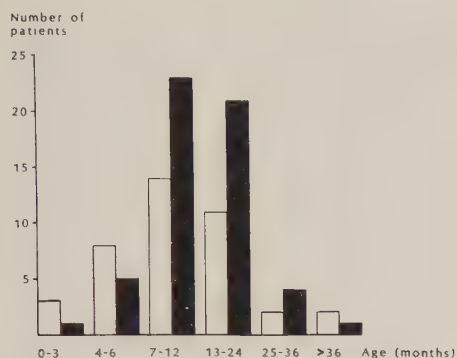


Fig. 2. Age distribution of patients with diarrhoea. □, rotavirus-negative patients; ■, rotavirus-positive patients.

Distribution of sex was equal among patients. According to Fig. 2, 91% were under two years of age (median age 11 months, range 1 month–4 years).

Clinical findings

Fig. 3 shows the presenting clinical symptoms. Acute symptoms such as fever, vomiting, watery diarrhoea and dehydration were more common with rotavirus infections. Associated respiratory symptoms were, however, not more common with rotavirus patients. Median duration of symptoms before hospital contact was 2 days (range 1–20 days). Seven patients had protracted diarrhoea more than 14 days. Duration of symptoms before and after hospital contact was not correlated to aetiology.

Macroscopic blood in stools was only observed in patients with bacterial aetiology—one with *Salmonella*, two with *Campylobacter*. A mild dehydration was found in four of the children with *Campylobacter* infection and the patient with *Salmonella*. Otherwise, the presenting clinical symptoms of patients with bacterial pathogens did not differ from other patients.

Treatment

Treatment was generally symptomatic, and specific antimicrobial therapy was only given

to four patients—the two with *Yersinia* infection, one with *Campylobacter* and one with unknown aetiology. Antibiotics were given because of proved (or in one case suspected) bacterial aetiology when the clinical response was poor after 2–3 days of initial intravenous rehydration therapy. Oral rehydration was generally successful and only in ten (11%) of the patients was i.v. therapy needed; rotavirus—four, *Campylobacter* + rotavirus—one, *Campylobacter*—two, *Yersinia*—two, unknown aetiology—one. The median duration of hospital care was three days (range 1–15 days).

DISCUSSION

Children presenting with symptoms of gastroenteritis such as fever, vomiting and diarrhoea give very few discriminating signs regarding the aetiology of the disease. The difference between rotavirus patients and others in our study was, in this aspect, not impressive. Abdominal pain is difficult to evaluate in small children. Other clinical signs are probably more related to the age of the patients than to the aetiological agent. Macroscopic blood in stools was a rare finding, only seen in

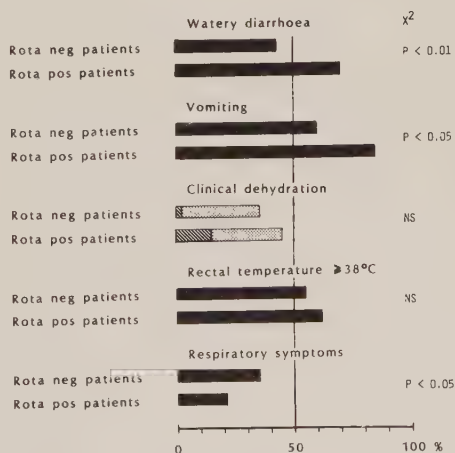


Fig. 3. Clinical signs and symptoms of patients at hospital contact (%). Clinical dehydration: ■, moderate; ▨, mild.

three patients, two of them with *Campylobacter* infection. For children under two years of age presenting with bloody diarrhoea and abdominal pain the differential diagnosis of *Campylobacter* infection versus intussusception must be kept in mind (12).

In epidemiological investigations of diarrhoea in children some years ago there was generally a low isolation rate of intestinal pathogens (13). After the discovery of rotavirus, it was shown that this agent was the most common cause of childhood diarrhoea both in industrial and developing countries (2, 14, 15).

The monthly distribution of diarrhoea found in this study corresponds with earlier observed winter-peaks of rotavirus infection (16, 17). Previous perennial studies indicate that rotavirus causes 28–65% of the hospitalized cases of childhood diarrhoea in temperate areas (17, 18, 19), compared to the 58% found in Malmö.

Clinical findings such as fever, vomiting, watery diarrhoea and dehydration are commonly seen, although the disease is generally mild in temperate areas (16, 17, 19, 20). Respiratory symptoms, however, did not correlate to rotavirus in this study. In contrast Lewis et al. reported respiratory symptoms in two third of rotavirus cases (21).

In contrast to rotavirus (58%), bacterial enteropathogens were comparatively rare (11%) among inpatients. In comparison, from November 1978 through July 1979 bacterial pathogens were detected in only 6% of 82 outpatients (unpublished data). It is of interest that two out of three inpatients infected abroad had bacterial pathogens. In addition the two non-domestic cases of the 82 outpatients both had bacterial enteropathogens—one EPEC O26:B6 the other *Campylobacter* + EPEC O126:B16 (unpublished data).

Campylobacter as a cause of diarrhoea is found in all age groups but according to Butzler especially in infants under two years of age (22). In developing countries high relative frequencies of *Campylobacter* infections

have been reported for infantile diarrhoea, 13–35% (15, 23). In industrialized countries corresponding frequencies of 4–6% have been reported from Belgium and Canada (24, 25). In a recent Swedish study including 435 patients with *Campylobacter* infection, 11% were under two years of age. The relative frequency of *Campylobacter* enteritis was 7% (5). *Campylobacter* is thus the most common bacterial agent associated with infantile diarrhoea in Sweden. In general, EPEC infections nowadays seem to be mild and rare in industrialized countries in contrast to countries with poor sanitary conditions (26). However, in a recent study from Finland, EPEC was still the most frequent bacterial pathogen encountered (3). The pathogenesis of EPEC diarrhoea remains obscure, and our isolates were negative concerning enterotoxin production and invasive capacity. Patients were not investigated for presence of enterotoxin producing strains belonging to other serogroups (ETEC). However, ETEC strains seems to be rare in Scandinavia (3, 27).

In these Swedish children, having a good state of nutrition, the clinical symptoms of diarrhoea were comparatively mild. Rehydration therapy could generally be administered orally and few patients received antibiotics. Due to the rather nonspecific clinical signs of infantile gastroenteritis, stool sampling is essential, in order to establish the local epidemiological pattern and to identify the aetiology of those critically ill patients who would benefit from specific antibiotic therapy.

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Enteropatogena *E. coli* — en anmälningspliktig sjukdom?

Epidemisk spädbarnsdiarré orsakad av enteropatogena E. coli (EPEC) var en fruktad sjukdom för ett par decennier sedan. Nyupptäckta fall av EPEC anmäls fortfarande enligt smittskyddslagen. En retrospektiv granskning av EPEC:s nuvarande roll i Sverige redovisas. Den nu praktiserade rutinmässiga anmälningen av EPEC utan hänsyn till klinik och smittspridning torde vara överflödig.

Under tidigt 1900-tal uppmärksammades epidemiska utbrott i Europa av spädbarnsdiarré, ofta i så svår form att den kallades »cholera infantum». Associationen till vissa stammar av *Escherichia coli* blev successivt klarlagd inte minst genom Brays arbete 1945 där man kunde visa att en viss serotyp av *E. coli*, benämnd *Bacterium coli neapolitanum*, var ansvarig för ett långvarigt epidemiskt utbrott av spädbarnsdiarré i England [1]. Denna serotyp har sedermera visats vara identisk med 0 111:B4, känd som »klassisk» enteropatogen *E. coli* (EPEC). Från 1940-talet och framåt påvisades ytterligare en rad serotyper som orsak till epidemiska utbrott och man har så småningom fastställt en lista med drygt tio serotyper under den gemensamma beteckningen EPEC.

Revisen för dessa kolistammars virulens är fortfarande endast epidemiologiska och hittills har man inte med laboratoriemetoder lyckats kartlägga infektionens patogenes. Toxinproduktion och invasiv förmåga hos *E. coli* är visserligen associerad till ett begränsat antal serotyper som dock *icke* sammanfaller med de »klassiska». Ett exempel på begreppsförvirring är serotyp 0 78 som i Sverige är listad bland de klassiska enteropatogena typerna men i internationella sammanhang brukar återfinnas bland toxinbildande stammar.

De epidemiska utbrott på institutioner för barnvård som förekom även i Sverige under 1940- och 1950-talen har studerats av bl a Laurell [2] som i sin avhandling anvisade metoder för behandling och begränsning av smittspridning. En övervakning på nationell nivå pågår sedan 1946.

Epidemisk spädbarnsdiarré är fortfarande anmälningspliktig enligt smittskyddslagen under rubriken »elakartad gastroenterit hos barn under två års ålder». Denna rubrik i smittskyddslagen talar sålunda ej om etiologi och skulle därför kunna vara tillämplig på t ex elakartade diarréer orsakade av rotavirus. Anmälningsplikten har emellertid tillämpats endast vid förekomst av EPEC i feces oavsett klinik och smittspridning.

EPEC är fortfarande en viktig orsak till framför allt endemisk barndiarré i utländer [3]. I industriländerna har dock de allvarliga utbrott med hög mortalitet på institutioner som rapporterades under 1940- och 1950-talen blivit alltmera sällsynta. I England inträffade ett av de senaste allvarliga utbrotten i Manchester 1968–1969 då 21 av 29 smittade barn krävde intravenös behandling och sju dog [4].

Ett annat epidemiskt utbrott med mortalitet på barninstitution beskrevs från Holland 1980 (Gerards et al, opubl data 1981) och visar att EPEC fortfarande är kapabel att framkalla diarréepidemier även i industriländerna.

Denna artikel vill värdera relevansen av EPEC i Sverige inför en planerad revision av smittskyddslagen.

Metoder

Statistiskt material från Statens bakteriologiska laboratorium över anmälda fall från 1946 och framåt har jämförts med spädbarnsmortalitet under samma period. Mellan åren 1975 och 1980 har fall anmälda enligt smittskyddslagen korrelerats med den frivilliga laboratorierapporteringen totalt och länsvis. Kliniska och epidemiologiska data från blanketter för anmälan enligt smittskyddslagen har evaluerats. Journaler för ett års fall anmälda enligt smittskyddslagen har analyserats. Samtliga barnkliniker och bakteriologiska laboratorier har tillskrivits med förfrågan om synpunkter på diagnostik och anmälan samt eventuell förekomst av epidemier. Arbetet har utförts i samarbete med epidemiologiska avdelningen, SBL.

Resultat

Bristfällig överensstämmelse EPEC-infektion—spädbarnsmortalitet

Figur 1 visar det totala antalet anmälda fall av EPEC-gastroenterit från 1946 till 1979. Som en jämförelse visas också den totala mortaliteten i diarré hos barn under ett års ålder (uppgifter från statistiska centralbyrån). Mortalitetskurvan visar en successiv avtrappning för att de senaste åren nå mycket låga tal. Överensstämmelse med antalet anmälda fall av EPEC-gastroenterit föreligger inte.

Diskrepans mellan anmälan enligt smittskyddslag och laboratorierapportering

Figur 2 visar en jämförelse mellan frivillig laboratorierapportering och anmälan enligt smittskyddslagen under 1975–1980. Parallellt med den totala minskningen av antalet anmälda fall ser man en tydlig diskrepans i förhållandet mellan laboratorieanmälda fall och rapporter enligt smittskyddslagen. Observera att den frivilliga laboratorierapporteringen upphörde från och med 1980. Länsvis fördelning av anmälningar enligt smittskyddslagen framgår av Figur 3.

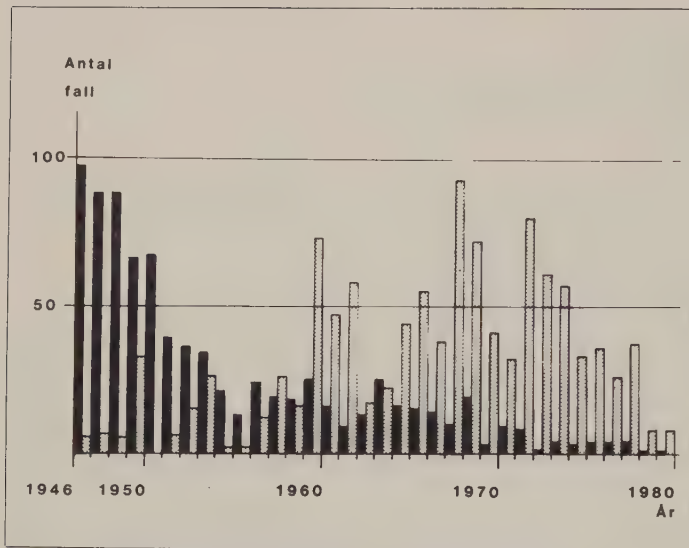
Endast X-län uppvisar något som skulle kunna likna en epidemisk anhopning framför allt 1975 med 17 anmälda fall enligt smittskyddslagen och 34 anmälningar från laboratoriet i Gävle. Enligt laboratoriet förelåg också en viss epidemisk anhopning som sannolikt hade samband med barndaghem. Ett flertal serotyper var då inblandade.

I övrigt skyllar vissa län, exempelvis G, S, U och W konsekvent utan anmälda fall. Man kan misstänka att detta snarare speg-

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Figur 1. ■ Mortalitet för barn under 1 år i diarré (enligt statistiska centralbyrån). ▨ Elakartad gastroenterit hos barn under 2 år. Anmälningar enligt smittskyddslagen.

lar aktiviteten i diagnostik och anmälning än verkliga epidemiologiska skillnader.

Informationsvärdet av smittskyddsbilletter

Evaluering av kliniska respektive epidemiologiska data på smittskyddsbilletterna åren 1975–1980 gav följande utbyte: totalt anmälades under denna period 129 patienter. Av dessa vårdades 64 i slutenvård, 61 i öppen vård (uppgift saknas om 4 patienter). Klinisk information förelåg om 72 patienter, varav 28 rörde hälsokontroll av adoptivbarn. En patient rapporterades som svårt sjuk. Epidemiologiska data förelåg i 68 fall, 47 av dessa gällde adoptivbarn eller utlandssmitta. Misstänkt spridning rapporterades i 8 fall varav familje-

smitta 3, nosokomialt förvärvat smitta 3 och familje + institutionssmitta 2.

Detaljanalys av ett års anmälda fall

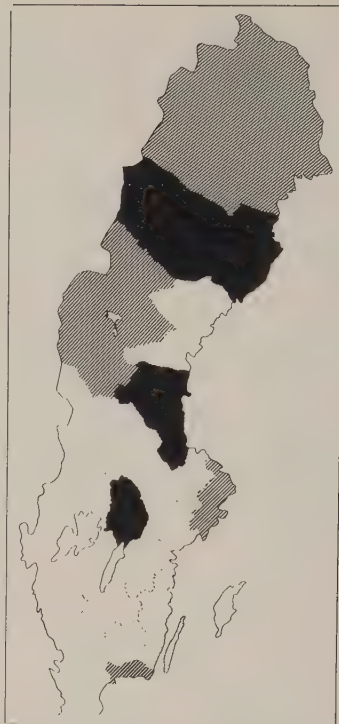
Tabell I redovisar en sammanfattning av totala antalet patienter som anmälts enligt smittskyddslagen under ett år – från och med juli 1980 till och med juni 1981. Av totalt 13 anmälda patienter hade många samtidigt fynd av andra tarmpatogener eller misstänkt födoämnesintolerans, EPEC-infektion kvarstod som huvudsakligt misstänkt agens endast hos fyra patienter. Den kliniska informationen synes ge vid handen att dessa barndiarréer knappast utmärkt sig på något särskiljande sätt jämfört med andra gastroenteriter hos barn. Av de epidemiologiska uppgifterna framgår att antalet utländska adoptivbarn är överrepresenterat, rimligen beroende på mer frikostig riktad provtagning och diagnostik i denna patientgrupp. Misstänkta sekundärfall förelåg hos två anmälda barn men inga allvariga sekundärfall och ingen nosokomial spridning.

Serotyper

13 olika serotyper är representerade mellan 1975 och 1979. De vanligaste är 0 55, 0 78, 0 86, 0 125 och 0 127. Fördelningen av serotyper totalt respektive länsvis ger dock heller inget epidemiologiskt mönster.

Synpunkter från barnläkare och bakteriologer

Mot bakgrund av den splittrade bild som framkommit beträffande EPEC-epidemiologin utsändes en enkät till barnkliniker och bakteriologiska laboratorier. 50 svar har kommit in från barnkliniker (respektive motsvarande regionala centra i frånvaro av större barnkliniker). 17 svarande rapporterar någorlunda regelbunden feces-provtagning med frågeställningen EPEC. Endast hos sex av dessa görs



Figur 3. Totala antalet sjukdomsfall 1975–1979 per 100 000 invånare (totalbefolkning). □ <1, ▨ 1–<2, ▩ 2–4, ■ >4.

dock rutinmässig provtagning på diarré-barn under två års ålder. Övriga indikationer är: svårt sjuka – terapieresistenta fall (16 svarande), adoptivbarn, invandrare (6 svarande), epidemi – misstanke om institutionssmitta (8), neonatal gastroenterit (7).

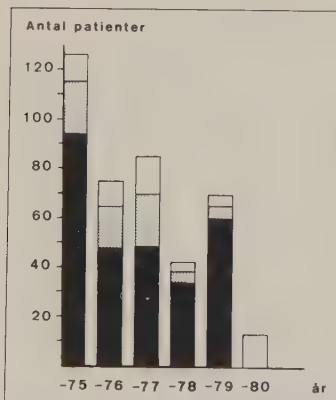
Barn med allvarlig klinik som tillskrivits enteropatogena *E. coli* under senaste året beskrevs i tre svar av 50. Ett av dessa barn fanns på S:t Görans barnsjukhus; övriga fanns på Sachsska barnsjukhuset/Huddinge sjukhus (se nedan). Två av 50 svarande rapporterade förekomst av epidemi senaste året (Sachsska/Huddinge).

Rutinmässig anmälning av EPEC enligt smittskyddslagen görs av 19 svarande medan 27 svarar att de ej rutinmässigt brukar anmäla sådana fall. På frågan om huruvida man anser nuvarande anmälningsplikt befogad svarar 9 entydigt »ja» och 24 entydigt »nej», övriga är tveksamma.

Av enkät till bakteriologiska laboratorier, varav 26 svar inkommit, framgår att diagnostiken bedrivs sparsamt. Samtliga laboratorier tillämpar rekommenderat förfarande, dvs objektglasagglutinerande stammar verifieras med kokning – titrering på eget laboratorium eller SBL. Flertalet laboratorier använder sera av SBL:s fabrikat.

EPEC-epidemi under 1981

Efter rundskrivelser framkom att epidemiisk spridning med enteropatogena *E.*



Figur 2. Fall anmälda som »elakartad gastroenterit hos barn under 2 års ålder». ■ Anmälda enligt frivillig laboratorierapportering (upphörde 1980). ▨ Anmälda enligt smittskyddslagen. □ Anmälda enligt båda ovanstående.

coli förekom juni–juli 1981 vid Sachsska barnsjukhuset. Patienter därifrån flyttades i samband med sommarstängning över till Huddinge sjukhus. Ansvariga läkare meddelar att det första fallet konstaterades den 25 juni hos ett barn som lades in för gastroenterit. Hon hade EPEC serotyp 0 111:B4. Denna patient synes ha gett upphov till sammanlagt tre sekundärfall. Ett prematurt fött barn som återintogs den 7 juli och lades in på prematuravdelning befanns ha EPEC 0 114. Man misstänker att denna patient gav upphov till nio sekundärfall som var symtomgivande. Av dessa avled två barn med Downs syndrom. Tarminfektionen synes åtminstone i ena fallet ha varit starkt bidragande. Under samma period inlades också ett barn med serotyp 0 26:B6 och sammanfattningsvis har alltså viss nosokomial spridning skett men under perioden har också flera av barnen förvärvat smittan utanför sjukhus. På tre av fallen prövades sulfonamid+trimetoprim (Bactrim), enligt uppgift utan framgång.

Diskussion

Genom tillkomsten av serotypning har det klart kunnat visas att vissa stammar av *E. coli* givit upphov till diarré hos små barn. Förekomst av EPEC i feces kunde ha en allvarig innebörd såväl för den enskilde patienten som för mottagliga kontakter särskilt på institutioner. Mot bakgrund av de ofta elakartade epidemier som förekom under 1940- och 1950-talen var det naturligt att instifta en epidemiologisk övervakning, och ur detta perspektiv får man se den anmälningsplikt som fortfarande gäller.

Flera klassiska infektionssjukdomar har med tiden ändrat karaktär, och det allmänna intrycket är att EPEC-infektion i industriländerna numera sällan har samma allvarliga innebörd som för ett par decennier sedan. En retrospektiv genomgång av statistiska uppgifter respektive anmälningsblanketter och journaler är alltid vanskelig, särskilt med hänsyn till ofull-

ständigheter i det tillgängliga materialet. Prospektiva undersökningar kan ytterligare belysa frågan om huruvida EPEC-infektioner intar en särställning jämfört med andra barnenteriter. Sådana undersökningar har också utförts eller pågår på flera håll i Norden [5, 6]. Ett dåligt utbyte av den nuvarande tillämpningen av smittskyddslagen kan dock i sig motivera en omprövning.

Av de data som presenteras i denna sammanställning kan man konkludera: Under drygt 30 års observationstid föreligger ingen påtaglig samvariation mellan anmälda fall av EPEC-gastroenterit och mortaliteten i gastroenterit hos barn under ett års ålder, vilket borde kunna förväntas med hänsyn till den rapporterade höga mortaliteten i tidigare epidemiska utbrott. Det finns en påtaglig diskrepans mellan fall anmälda enligt frivillig laboratorierapportering och fall anmälda enligt smittskyddslagen, varför de senare sannolikt endast är »toppen av ett isberg». Intrycket stärks av att intresset för EPEC-diagnostik och anmälning uppenbarligen varierar geografiskt i landet.

Man kan inte spåra några epidemier med hjälp av de upplysningar som de senaste fem åren lämnats på smittskyddsblanketterna. Detta gäller även det mindre utbrottet 1981 på ett sjukhus i Stockholm. Vid en direkt förfrågan till berörda barnkliniker har knappast några fall av epidemisk spridning rapporterats ens från ställen som bedriver en rutinmässig diagnos-tik.

En systematisk genomgång av ett års anmälda fall ger ett totalantal av endast 13. I ett fåtal av dessa fall kan EPEC med rimlig sannolikhet vara den primära orsaken till diarrén. En klar överrepresentation av adoptivbarn, ofta symptomfria, föreligger genomgående, möjligen beroende på riktad provtagning.

Då erfarenheter från utlandet samt från Sverige sommaren 1981 visar att enteropatogena *E. coli* fortfarande är kapabla att framkalla allvarig sjukdom och epidemisk spridning bör möjlighet till diagnostik finnas kvar. Den roll EPEC intar i u-län-

derna kan motivera fortsatt riktad provtagning vid diarré hos adoptivbarn eller vid utlandssmitta. EPEC-diagnostik bör även tillämpas vid allvarliga fall av gastroenterit hos barn med tanke på möjligheten att ge antibiotikabehandling [7]. Även fall av epidemisk spridning av diarré inom institutioner för barnavård bör föranleda provtagning och vid misstanke om epidemisk spridning bör givetvis en anmälan ske till smittskyddsmyndighet. Den anmälan som i dag sker på såväl symptomfria som måttligt sjuka barn med påvisad EPEC i feces och utan tendens till epidemisk spridning synes dock mogen att avskaffas.

Tabell I. Fall av *E. coli*-gastroenterit anmälda enligt smittskyddslagen 1980-07–1981-06.

Totalt antal anmälda patienter	13
Etiologi	
Patienter med samtidigt fynd av andra tarmpatogener	6
Salmonella 2, Campylobacter 2, <i>C. difficile</i> 1, Shigella + Giardia 1	
Antal patienter med misstänkt födoämnesintolerans	3
Övriga fall utan andra påvisade patogener eller födoämnesintolerans	4
Vårdform	
Inneliggande	11
Poliklinisk	2
Klinik	
Kronisk diarré = > 14 dagars anamnes	4
Klinisk dehydrering vid ankomst till sjukhus	3
varav i.v. behandling 1	
Antibiotikabehandling av diarrésjukdom	0
Lindrig klinik eller symptomfria	6
Epidemiologi	
Utländska adoptivbarn	4
varav symptomfria 2	
Misstänkt smittkälla sekundärspridning	2
varav barndaghem 1, intrafamiljär 1	
Nosokomial spridning eller allvarliga sekundärfall	0
Inhemsk infektion utan känd spridning	7

Litteratur

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Summary

Enteropathogenic *E. coli* — a notifiable disease

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Epidemic infantile diarrhoea caused by enteropathogenic *E. coli* (EPEC) was a serious health problem in Europe a few decades ago. In Sweden, new cases of EPEC are still reported according to Health Regulations. A retrospective epidemiological survey is presented which demonstrates an obvious decline in the importance of EPEC as an enteropathogen in Sweden. The current procedure for reporting EPEC irrespective of clinical and epidemiological circumstances is questioned.

(English translation)

ENTEROPATHOGENIC E. COLI - A NOTIFIABLE DISEASE?

Since the beginning of this century epidemic outbreaks of infantile diarrhoea have focused interest in Europe. The disease was often severe, attracting the name "cholera infantum". Several investigations indicated an association to certain strains of Escherichia coli. Bray (1945) incriminated a serotype of E. coli called Bacterium coli neapolitanum, BCN, as responsible for a protracted epidemic outbreak of infantile diarrhoea in England (1). This serotype was later found to be identical with 0111:B4, known as a "classical" enteropathogenic E. coli (EPEC). Since the 1940s, more serotypes of E. coli were associated with epidemic outbreaks of infantile diarrhoea finally resulting in a list of more than ten recognized EPEC serotypes.

The proofs of the virulence of EPEC are still only epidemiological and the pathogenesis of infection is still obscure. Enterotoxin production and invasive ability in E. coli are indeed associated with a limited number of serotypes. However, these serotypes generally do not coincide with the "classical" ones. Serogroup 078 exemplifies the confusion in present terminology: in Sweden it is listed among EPEC strains but in international literature it is usually listed among enterotoxigenic strains.

Epidemic outbreaks occurring in paediatric institutions in Sweden during the 1940s and 1950s have been studied by Laurell (2), who described methods for therapy and epidemic control. A national epidemic surveyance of EPEC has been performed since 1946.

Epidemic infantile diarrhoea is still a notifiable disease according to health regulations* under the name: "Severe gastroenteritis of children under 2 years of age". This paragraph in the health regulations does not

*This duty of reporting refers to clinical centra. Laboratory reports of clinical isolates are voluntary and independant.

precise any aetiology of the disease and should thus be applicable to - for instance - severe diarrhoea caused by rotavirus. However, this paragraph has only been applied on the occurrence of EPEC in stools irrespective of clinical disease or epidemiological circumstances.

EPEC are still an important cause of infantile diarrhoea in developing countries, particularly in endemic disease (3). In industrialized countries, however, the serious epidemic institutional outbreaks with a high mortality reported during the 1940s and 1950s have become increasingly rare. In England, one of the latest serious outbreaks occurred in Manchester 1968-1969. Twenty-one out of 29 affected children needed intravenous therapy and seven died (4).

Another epidemic outbreak resulting in deaths was reported from a paediatric ward in Holland 1980 (Gerards et al unpublished data 1981). This shows that EPEC are still capable of causing epidemics of diarrhoea also in industrialized countries.

The object of the present investigation is to evaluate the relevance of EPEC infections in Sweden prior to a planned revision of health regulations.

METHODS

Statistical data from the National Bacteriological Laboratory concerning reported cases since 1946 were compared with infant mortality rates during the same period. Cases reported according to health regulations 1975-1980 were correlated with the reports of EPEC isolates from bacteriological laboratories. The correlation was done regarding both national and regional (county) level. Clinical and epidemiological information has been evaluated from individual forms of reports according to health regulations (1975-1980). Hospital records of cases, reported according to health regulations, during one year were analyzed. Inquiries have been sent to all paediatric clinics and bacteriological laboratories regarding opinions of diagnostic activity, the present report regulations and any occurrence of epidemics. The investigation was performed in cooperation with the Epidemiological Department, National Bacteriological Laboratory.

RESULTS

Lack of correlation, EPEC infections - Infant mortality

Figure 1 shows the total number of reported cases of EPEC infections 1946-1979. Infant mortality (under one year of age) in diarrhoea is shown for comparison. (Data from the National Central Bureau of Statistics). Mortality cases show a gradual decrease in number arriving at a very low number of cases in later years. There is no accordance with the number of reported EPEC infections.

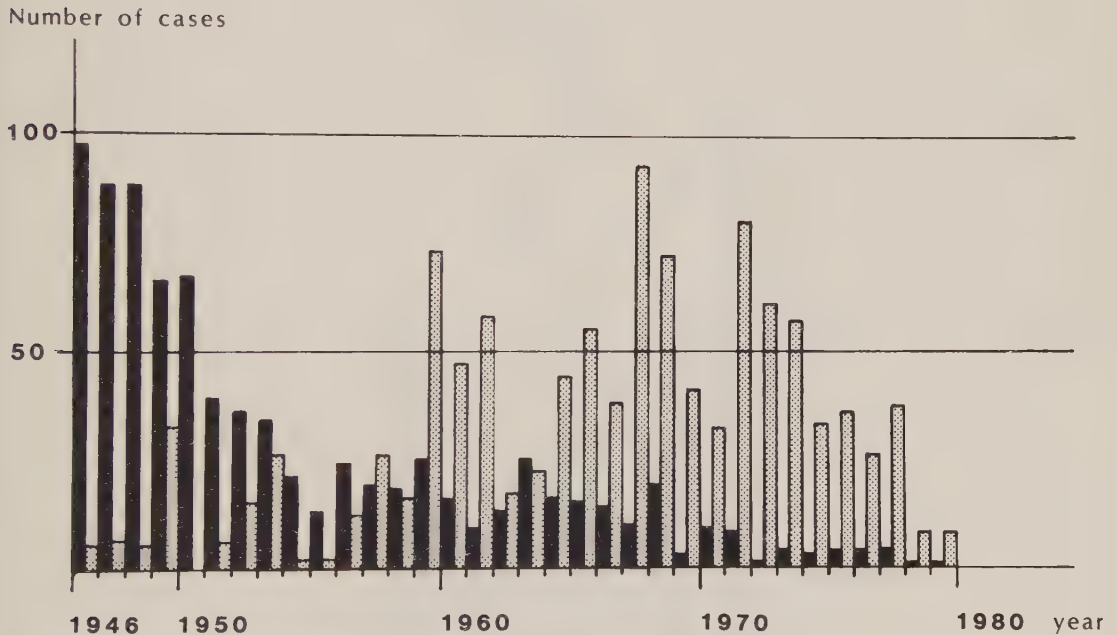


Figure 1. ■ Infant mortality (<1 year of age) in diarrhoea (according to the National Central Bureau of Statistics). ▨ "Severe gastroenteritis of children under 2 years of age". (Reports according to health regulations).

Disaccordance - reports according to health regulations versus laboratory reports of isolates

Figure 2 shows a comparison between the voluntary laboratory reports and the reports according to health regulations 1975-1980. Beside a total decrease in number of reported cases there is an obvious disaccordance between the two independent sources of reports. (Note that laboratory reports ceased 1980). Distribution of reports per county is shown in Figure 3.

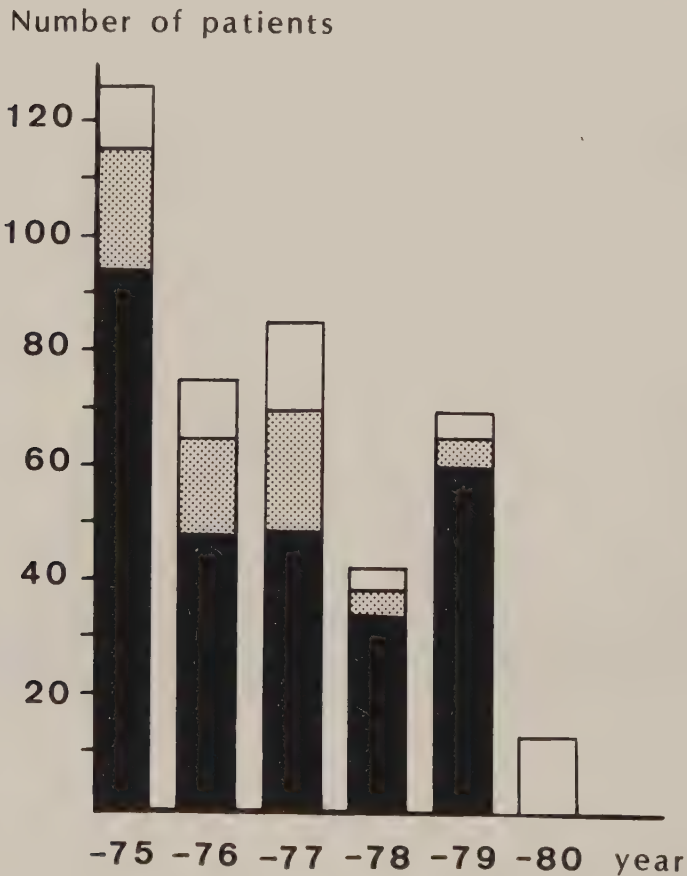


Figure 2. Number of cases reported as "severe gastroenteritis of children under 2 years of age". ■ Laboratory reports (ceased in 1980). □ Clinical reports according to health regulations. ▨ Reports according to both the previous sources.



Figure 3. Total number of EPEC cases 1975-1979 per 100 000 inhabitants (total population).

□ <1, ▨ 1-2, ▩ 2-4, ■ >4.

Only the county of Gävleborg (X) presents what might be called an epidemic accumulation of cases, particularly in 1975 with 17 cases according to health regulations and 34 reports from the bacteriological laboratory in Gävle. According to the laboratory, there was indeed a certain epidemic accumulation that probably had a connection with day-nurseries. A number

of different serotypes were involved.

Otherwise, some counties for instance G, S, U, and W consistently show off with no reported cases. It is assumed that this fact more reflects the activity of diagnostics and reporting than true epidemiological differences.

The quality of information in forms of report

The evaluation of clinical and epidemiological data in the forms of report 1975-1980 gave the following results: In all 129 patients were reported, 64 of them were hospitalized, 61 outpatients (data are missing in four patients). Clinical information was given in 72 reports - out of these 28 were health check-ups of adopted children from abroad. One patient was reported severely ill. Epidemiological data were given in 68 reports, 47 of these concerned adopted children or import cases. Suspected secondary cases were reported in eight forms: intrafamiliar - 3, nosocomial - 3, intrafamiliar + institutional - 2.

Detailed analysis of cases reported during one year

Table I summarizes all patients reported due to health regulations during one year - July 1980 through June 1981. Out of 13 reported patients with EPEC infection many had concomitant findings of other enteropathogens or suspected food-intolerance. EPEC infection remained the principal suspected agent in only four patients. Available clinical data indicate that the disease in these children was hardly distinguishable compared with other cases of gastroenteritis in children. From available epidemiological information it is obvious that the number of adopted children from other countries is overrepresented. This is probably due to a more active diagnostic policy versus this group. Suspected secondary cases were reported in two cases but no serious one and no nosocomial spread of disease.

Table 1. Cases of EPEC disease reported according to Health regulations July 1980 through June 1981.

Total number of cases	13
<u>Aetiology</u>	
Patients with concomitant findings of other enteropathogens	6
Salmonella 2, Campylobacter 2, Cl difficile 1, Shigella + Giardia 1	
Patients with suspected food intolerance	3
Remaining cases	4
<u>Status_of_Patients</u>	
Admitted	11
Outpatients	2
<u>Clinical_data</u>	
Chronic diarrhoea (>14 days)	4
Dehydration at admission to hospital (One patient received i.v. treatment)	3
Antibiotica therapy for the diarrhoea	0
Mild or no symptoms	6
<u>Epidemiology</u>	
Adopted children from non-European countries (2 without symptoms)	4
Indications of secondary cases (one day-nursery, one intra-household)	2
Nosocomial spread or serious secondary cases	0
Domestic cases without indications of secondary cases	7

Serogroups

Thirteen different serogroups were represented 1975-1979. Most common were 055, 078, 086, 0125, and 0127. Distribution of serogroups totally or geographically did not give any epidemiological pattern.

Opinions from paediatricians and bacteriologists

Due to the fragmentary view of EPEC epidemiology that had appeared so far, an inquiry was sent to paediatric institutions and bacteriological laboratories. Fifty inquiries were answered by paediatric institutions. Seventeen of them report a reasonably regular stool sampling for EPEC. However, only six of them perform a routine screening for EPEC in children under two years of age with diarrhoea. Other reasons for EPEC-sampling were: Severely ill - therapy resistant cases - 16, adopted children - immigrants - 6, epidemic - suspected institutional spread - 8, neonatal gastroenteritis - 7.

Children with severe clinical disease associated with EPEC were reported in three answers out of 50. One of these children was admitted to St Görans Hospital, Stockholm, the others to Sach's and Huddinge hospitals (see below). Two out of 50 answers reported epidemic spread within the last year (Sach's and Huddinge).

Routine notifying of EPEC according to health regulations was performed by 19 out of 50 institutions, 27 did not. Regarding personal opinion on the relevance of the present notifying obligation nine were clear-cut positive, 24 clear-cut negative and the rest undecided.

Inquiries to bacteriological institutions were answered by 26. It was obvious that the identification of EPEC is performed sparingly. All laboratories applied the recommended procedure meaning that isolates positive in slide agglutination are confirmed by heating and tube-agglutination locally or at the National Bacteriological Laboratory (NBL). Most laboratories use antisera manufactured by NBL.

Epidemic of EPEC during 1981

As a result of inquiries it was obvious that an epidemic spread of EPEC occurred June-July 1981 at the Sach's Children Hospital in Stockholm. Some children with EPEC diarrhoea were later transferred to Huddinge hospital due to planned summer vacations. According to doctors in charge, the first case of EPEC diarrhoea (serotype 0111:B4) was confirmed 25 June. She was admitted because of gastroenteritis, and seems to have caused three secondary cases. Another premature born child was readmitted 7 July to the neonatal ward. Later, the patient proved to be infected with EPEC 0114. It was believed to have caused nine additional secondary cases with gastroenteritis. Two of them, having Down's syndrome, died. At least in one case the gastroenteritis was decidedly contributive. During the same period a child having EPEC 026:B6 was admitted. In conclusion a nosocomial spread was established but during the period many children were infected outside the hospital. Trimethoprim-sulphamethoxazole (Bactrim) was tried on three cases with a doubtful result.

DISCUSSION

By the development of serotyping it was clearly shown that certain strains of E. coli caused infantile diarrhoea. The presence of EPEC in stools might have serious implications for the individual patient and susceptible contacts, particularly in institutions. In the light of the often serious epidemics that occurred during the 1940s and 1950s it was natural to establish an epidemiological surveyance, and the still valid health regulations should be viewed from this perspective.

The clinical appearance of many classical infectious diseases has changed in course of time, and a general impression tells that EPEC infections in industrialized countries nowadays rarely have the same serious implications as some decades ago. A retrospective analysis of statistical data, reports and hospital records is always problematic, particularly due to shortcomings in the available material. Prospective investigations would further elucidate the question whether EPEC infections hold an exceptional position compared with other infantile diarrhoeas. Such investigations have been

performed and are performed in many Nordic centra (5, 6). However, an insufficient outcome of the present application of health regulations may in itself justify a reconsideration.

From data presented in this investigation, the following conclusions are made: During more than 30 years of observation there is no obvious co-variation between the reported cases of EPEC-gastroenteritis and the mortality in gastroenteritis of children under one year of age. Such a co-variation might be expected due to the high mortality reported in earlier epidemic outbreaks. There is an obvious disaccordance between cases reported according to the voluntary laboratory reports and cases reported according to health regulations. It is assumed that the later cases only represent "the peak of an iceberg". This impression is fortified by the fact that activity in EPEC-investigation and - notifying obviously varies geographically in Sweden.

It was not possible to trace any epidemics by information given in report forms during the last five years. This is also true regarding the minor outbreak 1981 in a hospital in Stockholm. By direct inquiry to the paediatric institutions concerned it became clear that hardly any cases of epidemic spread were noted even from places performing routine screening for EPEC.

A methodical analysis of cases reported during one year included only 13 patients. In a minority of these cases EPEC were incriminated with a reasonable probability as a primary cause of the diarrhoea. Adopted children, often without symptoms were generally overrepresented, possibly due to selective sampling policy.

As experiences from abroad and from Sweden 1981 demonstrate that EPEC are still capable of causing severe disease and epidemic spread, a diagnostic potential should still be available. The role of EPEC in developing countries may justify continuous sampling in cases of diarrhoea in adopted children or import cases. Sampling for EPEC should also be carried out in severe cases of gastroenteritis in children due to the possibility of antibiotic treatment (7). Also in cases of epidemic diarrhoea in paediatric institutions EPEC sampling should be performed and - at suspected epidemic spread - a notifying be made to health authorities. However, the present policy of routine report in symptomless and moderately sick children having EPEC-

positive stool samples but no sign of epidemic spread seems ready to be abolished.

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Antibiotics in the Treatment of Gastroenteritis Caused by Enteropathogenic *Escherichia coli*

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The role of antibiotics in treating endemic infantile diarrhea caused by enteropathogenic *Escherichia coli* has not been determined. In a controlled study of 49 patients, one group received mecillinam and another group received trimethoprim-sulfamethoxazole. A third group served as control subjects. Serotype O111:B4 dominated. Treatment, as evaluated clinically on the third day, resulted in cure for 79% of those receiving mecillinam, 73% of those receiving trimethoprim-sulfamethoxazole, and 7% of the control subjects. Bacteriologic cure was confirmed in 53%, 53%, and 0, respectively. The statistically significant difference between antibiotic-treated patients and control subjects ($P < 0.001$) indicated that antibiotics are an important supplement in the treatment of endemic severe diarrhea caused by enteropathogenic *E. coli*.

Acute diarrheal diseases among children constitute a worldwide problem, especially serious in countries of Asia, Africa, and Latin America [1]. The incidence of gastroenteritis due to enteropathogenic *Escherichia coli* (EPEC) is reported to be 5%–10% in these developing countries, and studies from Canada also emphasize the role of EPEC as a cause of endemic infantile diarrhea [2–4]. Epidemiologic studies from Addis Ababa indicate that EPEC is a common cause of diarrhea [5–7]. Moreover, the clinical course of these infections is often debilitating. Although EPEC gastroenteritis has been recognized since the 1940's [8, 9], its pathogenesis is still obscure. The usefulness of serogrouping *E. coli* found in sporadic cases of diarrhea has been subject to debate [10, 11].

The frequent severity of the clinical course of EPEC diarrhea emphasizes the need for proper therapy. The use of antibiotics has been highly controversial [12], but very few clinical trials of

antibiotics that included untreated control patients have been performed for treatment of endemic EPEC gastroenteritis [13, 14]. The aim of this study was to determine whether severe EPEC gastroenteritis should be treated with antibiotics. We wanted to use nontoxic drugs with high efficacy against *E. coli*. Trimethoprim-sulfamethoxazole has been used successfully for treating enteric infections [15]. Mecillinam is also a nontoxic drug, highly effective against *E. coli* [16].

Materials and Methods

Clinical methods. Patients with diarrhea (defined as at least four watery stools per day) and in need of iv rehydration therapy were treated in a special rehydration unit at the Outpatients Department, Ethio-Swedish Pediatric Clinic, Addis Ababa, Ethiopia. Stool cultures were made by rectal swab; fresh stool samples were examined for cells and parasites by microscopy. After the culture report on the next day, EPEC-positive patients were admitted to a ward if they still needed hospital care. Children also infected with a second organism, such as *Salmonella*, *Shigella*, *Entamoeba histolytica*, or *Giardia lamblia*, and children treated with broad-spectrum antibiotics within the preceding three days were excluded from the study. Informed consent was obtained from the parents of each patient entering this study.

In the ward a clinical assessment was made, and the patients were classified according to age and

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sex, type of feeding, and nutritional status [17]. Duration of present diarrheal disease as well as the degree of dehydration, estimated clinically as mild, moderate, or severe [18] on admission to the rehydration unit, were recorded. Other concurrent diseases were noted.

Patients were randomly assigned to three groups. One group was treated with mecillinam (Leo Pharmaceutical, Copenhagen, Denmark), 40 mg/kg per day given in four im doses. Another group was given 6.4 mg of trimethoprim and 32 mg of sulfamethoxazole (Bactrim®; F. Hoffman-La Roche & Co. AG, Basle, Switzerland)/kg per day in four iv doses. Drug dosage was based on the manufacturer's recommendation. When the patient's clinical condition permitted, the drugs were given orally, in the first group as a piv-mecillinam solution at twice the parenteral dose, and in the second group as a trimethoprim-sulfamethoxazole mixture in the same dosage as

that given parenterally. Oral dosages were chosen so that serum concentrations corresponded to those obtained with parenteral administration. The antibiotics were given for five days, and studies on serum concentration were performed in selected cases. Patients assigned to the third (control) group were initially given no antibiotics but received the same supportive therapy with fluid and electrolytes as patients in the first two groups. Oral feeding was started when vomiting became less frequent.

The patients were examined daily for general condition, weight, pulse, rectal temperature, vomiting, number and consistency of stools, and suspected side effects of the drugs, such as exanthema or fever. The clinical follow-up was done by one investigator and a blind evaluation by another from the test protocols. Bacteriologic follow-up was done by daily stool cultures.

The criterion for clinically successful treatment

Table 1. Clinical characteristics of 49 children with diarrhea due to enteropathogenic *Escherichia coli* who received supportive therapy with fluids and electrolytes (controls) or supportive therapy combined with mecillinam or trimethoprim-sulfamethoxazole (TMP-SMZ).

Characteristic	No. treated with indicated characteristic		
	Mecillinam (n = 19)	TMP-SMZ (n = 15)	Controls (n = 15)
Age (months)			
0-3	5	6	7
3.5-6	7	3	5
6.5-12	5	5	2
12.5-24	2	1	1
Sex			
Male	11	8	8
Female	8	7	7
Feeding			
Breastmilk only	0	3	2
Breastmilk and supplement	10	9	7
Formula	9	3	6
Nutritional status			
Normal	6	6	5
Underweight	9	7	5
Kwashiorkor	0	0	1
Marasmus	4	2	3
Marasmic kwashiorkor	0	0	1
Duration of diarrhea before admission (days)			
3-7	7	5	10
8-14	7	7	3
>14	5	3	2
Degree of dehydration			
Mild	7	2	1
Moderate	7	9	11
Severe	5	4	3
Fever present	6	5	4

was a complete disappearance of watery stools within three days after the starting of treatment.

Laboratory methods. Each specimen was cultivated in the laboratory at the Ethio-Swedish Pediatric Clinic on deoxycholate citrate and MacConkey's agar.

Polyvalent and monovalent antisera were supplied by Dr. H. Rische, Institut für Experimentelle Epidemiologie, Wernigerode, German Democratic Republic. The antisera included serogroups O26:B6, O44:K74(L), O55:B5, O78, O111:B4, O114:K90, O119:B14, O124:B17, O125:B15, O126:B16, O127:B8, and O128:B12. The antisera thus contained serogroup O78, a strain only rarely listed as enteropathogenic [19].

Screening for EPEC was performed with use of polyvalent antisera by slide agglutination from the area of confluent growth on MacConkey's agar. When positive, single colonies were subcultured on MacConkey's agar and retested by slide agglutination with monovalent antisera. Strains were sent to Malmö General Hospital, the Department of Bacteriology, Sweden, and were confirmed by tube agglutination with use of a boiled suspension of organisms. Antisera from Wellcome Reagents Ltd., Beckenham, England, and reference strains supplied by Dr. Fritz Ørskov, State Serum Institute, Copenhagen, Denmark, were used for this procedure.

Statistical methods. Homogeneity in clinical background data and difference in treatment results were evaluated by χ^2 test.

Results

Clinical results. Clinical data for the 49 patients included in the study are presented in table 1. The three separately listed treatment groups were comparable. All patients were under two years of age (average age, four months), and 31 were breast-fed, usually in addition to formula feeding. Eleven of the 49 suffered from severe malnutrition, and 39 had moderate to severe dehydration on admission. Ten patients also had diarrhea of more than 14 days duration, but this was not correlated with the type of feeding or therapeutic result.

The following concurrent diseases were observed: otitis media (six patients), pneumonia (one), abscess (one), severe hypokalemia (one), and severe anemia (one). Penicillin was given in addition to other therapy in eight cases. Penicillin

Table 2. Therapeutic results in 49 children with diarrhea due to enteropathogenic *Escherichia coli* who received supportive therapy with fluids and electrolytes (controls) or supportive therapy combined with mecillinam or trimethoprim-sulfamethoxazole (TMP-SMZ).

Criteria	No. treated who met criterion (%)		
	Mecillinam (n = 19)	TMP-SMZ (n = 15)	Controls (n = 15)
Clinical cure within three days from start of therapy	15 (79)	11 (73)	1 (7)
Enteropathogenic <i>E. coli</i> eradicated from stools within three days*	10 (53)	8 (53)	0 (0)†

* Extending follow-up to five days on all patients on continued therapy (failed control patients excluded) yielded one further bacteriologic cure in each of the three groups.

† Stool culture follow-up was missed in three patients.

treatment and concurrent diseases were equally distributed among the three groups.

Bacteriologic results. Serogroup O111:B4 was the most common finding (31 patients). The other serogroups were: O26:B6 (two), O44:K74(L) (two), O55:B5 (three), O78 (five), O114:K90 (one), O119:B14 (three), O126:B16 (one), and O127:B8 (one). Strains from five patients were serogrouped only with pooled sera. Most patients had only one serogroup (44 patients). Four patients had two serogroups, and one had three different serogroups. The distribution of serogroups was equal among the three different treatment groups. Serogroup O78 is commonly found among enterotoxinogenic strains. Toxin production of the strains was assayed at the National Bacteriological Laboratory, Stockholm, Sweden. Toxin production (heat-labile and heat-stable) was confined to the five strains of serogroup O78.

MICs of all tested strains indicated full sensitivity to the two drugs within the range of the dosages used for treatment.¹ Stool microscopy showed few to moderate numbers of pus cells but no red blood cells.

Therapeutic results. Table 2 shows the results of therapy according to the criterion previously described. The difference between the groups given antibiotics and the control group was highly

¹ A. Thorén, "Antibiotic Sensitivity of Enteropathogenic *Escherichia coli* to Mecillinam, Trimethoprim-Sulfamethoxazole and Other Antibiotics," manuscript in preparation.

significant ($P < 0.001$). One patient died. He belonged to the control group and did fairly well for the first three days, but on the fourth day he suddenly developed profuse watery diarrhea and died.

The two groups tested with antibiotics were comparable in bacteriologic outcome of therapy, and the difference between these groups and the control group was significant ($P < 0.01$).

Side effects. One side effect attributable to treatment was observed: mild exanthema on the fifth day in a patient treated with trimethoprim-sulfamethoxazole.

Discussion

Diarrheal disease remains a dominant health problem in the pediatric population, particularly in developing countries. In Addis Ababa children younger than two years of age suffer from diarrhea on the average two months each year [20]. The majority of patients respond well to simple rehydration therapy and a dietary regimen. However, among these diarrheal patients, there remains a group often characterized by pronounced dehydration and long-term disease. Coetzee and Leary [21] found that this syndrome was associated with EPEC-positive stool cultures, a finding similar to our experience. Further study is required to determine the relative importance of EPEC gastroenteritis in endemic diarrhea as well as in selected groups of critically ill patients [1].

Although most authors agree on a nonantibiotic regimen for salmonella diarrhea [22], the proper treatment for EPEC gastroenteritis, especially endemic cases, is still under debate. The results of the very few controlled clinical trials with antibiotics are conflicting [13, 14]. One possible explanation could be that the severity of the disease was different in the groups studied. We found it justified to include an untreated control group and to restrict the study to patients in definite need of hospitalization after initial rehydration therapy.

The syndrome of gastroenteritis poses considerable difficulties in the evaluation of therapy. We found the presence of watery diarrhea among our patients to be closely associated with poor general condition. Diarrhea was defined as a minimum of four watery stools per day to avoid any error in interpreting a single stool as watery or not. Although the duration of therapy was five days [23], we chose the time for evaluation as

three days. Otherwise, hospitalization for the untreated control patients might have been prolonged unnecessarily.

Our results confirmed that EPEC gastroenteritis is restricted mainly to children younger than two years of age. Breast-feeding did not provide full protection. Only 18 of the 49 patients were exclusively formula-fed. Eleven of the children were severely malnourished, and the majority were below the 80th percentile of the Harvard standard [17]. Although the nutritional status of 17 patients was normal, the severity of their disease did not differ significantly from that of the others. Serogroup O111:B4 dominated in this study, and few patients had more than one serogroup.

When antibiotic therapy was given, the clinical response was usually prompt, within one to two days; it was often, but not always, correlated with the disappearance of EPEC from stools. Obviously a carrier state in the convalescent phase could be consistent with full clinical recovery, a possibility suggesting that EPEC is a disease of the small intestine [24]. This view has also been supported by the results of cultures from duodenal juice before and after treatment in seven patients, in whom complete disappearance of EPEC from the small intestine was correlated with clinical improvement.² Protracted diarrhea in children has been shown to be associated with coliform contamination of the small bowel [25]. Eradication of this flora by the antibiotics may also explain the favorable clinical response in all of the eight patients with protracted diarrhea treated with antibiotics.

Controlled studies on volunteers have demonstrated that a high bacterial load is required to establish EPEC gastroenteritis [26]. Most of our patients were from crowded environments with low socioeconomic standards, and, as a study from Gambia has shown, the food consumed by such children is highly contaminated by coliform bacteria [27]. In our study cultures on the contents of some of the feeding bottles showed massive coliform contamination.³

Cases of clinical failure with antibiotic therapy, with or without persistence of EPEC in stools,

² G. Stintzing and D. Habte, "The Small Intestinal Flora in *Escherichia coli* Diarrhea," manuscript in preparation.

³ See footnote 2.

could not be explained by in vitro resistance to the drugs used.⁴ In some cases a change of antibiotic therapy altered the course of disease. Other causes of diarrhea such as lactose intolerance could not be excluded.

We conclude that in hospitalized children younger than two years of age with proven EPEC diarrhea, general supportive therapy with fluid and electrolytes is inferior to a regimen that also includes antibiotic treatment. Although mecillinam and trimethoprim-sulfamethoxazole proved to be effective and safe in this study, the choice of antibiotic agents has not been settled and must be adapted to local conditions.

⁴ See footnote 1.

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ANTIBIOTIC SENSITIVITY OF ENTEROPATHOGENIC *ESCHERICHIA COLI* TO MECILLINAM, TRIMETHOPRIM-SULFAMETHOXAZOLE AND OTHER ANTIBIOTICS

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Thorén, A. Antibiotic sensitivity of enteropathogenic *Escherichia coli* to mecillinam, trimethoprim-sulfamethoxazole and other antibiotics. Acta path. microbiol. scand. Sect. B, 88: 265-268, 1980.

Forty-six clinical isolates of enteropathogenic *Escherichia coli* (EPEC) collected in Addis Ababa, Ethiopia, 1977-1978, were tested for susceptibility to 12 different antibiotics and beta-lactamase-production. Special reference was made to mecillinam and trimethoprim-sulfamethoxazole (TMP-SMZ) that were recently shown to be effective in the treatment of severe gastroenteritis caused by EPEC. Twenty-nine of the strains were of serotype 0111:B4. Thirty of the strains were resistant to 4 antibiotics or more, most of these strains belonging to serotype 0111:B4. For mecillinam, 19 strains had minimal inhibitory concentration (MIC) ≤ 0.2 $\mu\text{g/ml}$, 27 strains had MIC 0.8-3.2 $\mu\text{g/ml}$. Regarding TMP-SMZ, 41 strains had MIC ≤ 1 $\mu\text{g/ml}$, 5 strains had 2-4 $\mu\text{g/ml}$. No strain was resistant to gentamicin or nalidixic acid. Increased production of beta-lactamase was correlated to ampicillin resistance.

Key words: Antibiotic sensitivity; enteropathogenic *E. coli*; mecillinam; trimethoprim-sulfamethoxazole.

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The use of antibiotics in the treatment of diarrhoea caused by enteropathogenic *Escherichia coli* (EPEC) has been controversial especially in endemic cases of diarrhoea (8). Studies from different geographical areas have advocated their use (1, 2, 4, 9) and in consequence *in vitro* susceptibility studies of clinical isolates to different antibiotics have been performed (7, 10).

At the Ethio-Swedish Pediatric Clinic, Addis Ababa, Ethiopia, we performed a controlled clinical study of 49 patients with severe EPEC diarrhoea (12). The patients received either mecillinam, trimethoprim-sulfamethoxazole (TMP-SMZ) or no antibiotic treatment. The clinical response was very favourable, with a clinical cure rate of 79% for mecillinam treatment and 73% for TMP-SMZ, compared to 7% for the controls.

This investigation demonstrates *in vitro* susceptibility to mecillinam, TMP-SMZ and other antibiotics, thus giving possible alternatives of therapy for diarrhoea caused by EPEC.

MATERIALS AND METHODS

Source and Identification of Bacteria

EPEC isolates from 46 patients attending the Ethio-Swedish Pediatric Clinic, Addis Ababa, Ethiopia during 1977 and 1978 were included. Twenty-five of the isolates belonged to patients included in the clinical trial previously mentioned. All strains were isolated before antibiotic therapy.

The bacteria were identified as *Escherichia coli* using the API 20 E system (Analytab, New York, USA). Serotyping was performed by routine methods as previously described (12). The isolates consisted of the

following serotypes: 044:K74 (L) (4), 055:B5 (4), 0111:B4 (29), 0114:K90 (2), 0119:B14 (5), and 0126:B8 (2).

Assay of toxin production on all strains was performed at the National Bacteriological Laboratory, Stockholm, Sweden. No strain was toxigenic referring to heatlabile or heatstable toxin production.

Sensitivity Testing

The minimum inhibitory concentration (MIC) was determined for mecillinam and TMP-SMZ using the agar dilution method (5). Mecillinam was obtained from Leo Pharmaceutical, Copenhagen, Denmark, and TMP-SMZ from KABI, Sweden. The proportion of trimethoprim to sulfamethoxazole was 1:20, thus reflecting the serum ratio (3). *E. coli* AT CC 25922 was used as reference strain. For all other antibiotics sensitivity testing was performed according to the disc diffusion method (5). PDM medium, that was used for all determinations and antibiotic discs were purchased from Biodisk, Sweden. Breakpoints for susceptibility were in accordance to those of the Reference Group for Antibiotic Sensitivity Testing of the Swedish Medical Society's Sections for Medical Microbiology and Infectious Diseases. Breakpoints for neomycin were chosen according to Biodisk, Sweden, since neomycin is not yet included in the list of the reference group. The breakpoint for mecillinam is still only defined with reference to urinary tract infections while it is not used in this context.

Beta-lactamase Determinations

Estimations of beta-lactamase activity were kindly performed by Jens Keiding, Leo Pharmaceutical Products, Copenhagen, Denmark. Estimations were carried out according to a modification of the method described by Uri *et al.* (13). Briefly, the chromogenic cephalosporin nitrocephin was used as substrate. A change of colour,

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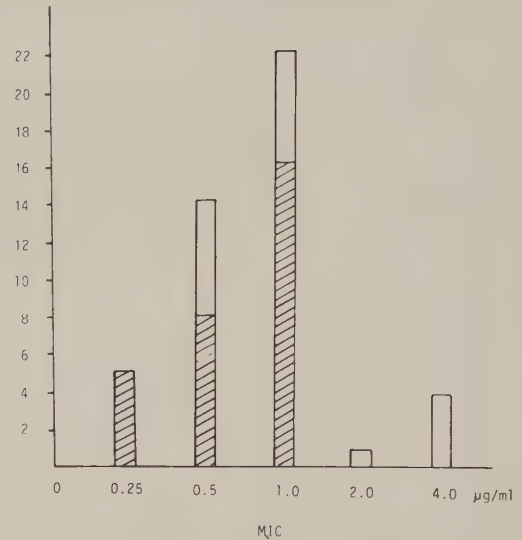


Fig. 2. MIC values for TMP/SMZ in weight proportions 1:20. Serotype 0111:B4 ▨. Other serotypes □.

when the substrate was exposed to suspensions of beta-lactamase producing bacteria, was read by visual inspection at appropriate intervals. One unit of activity is defined as the amount of enzyme which in one minute in volume of one ml converts the assay mixture from yellow to red.

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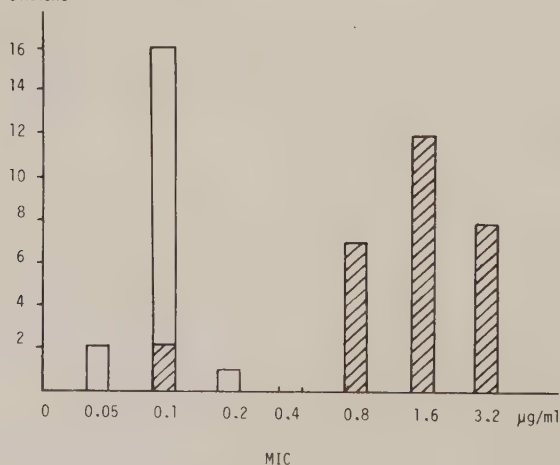


Fig. 1. MIC values for mecillinam. Serotype 0111:B4 ▨. Other serotypes □.

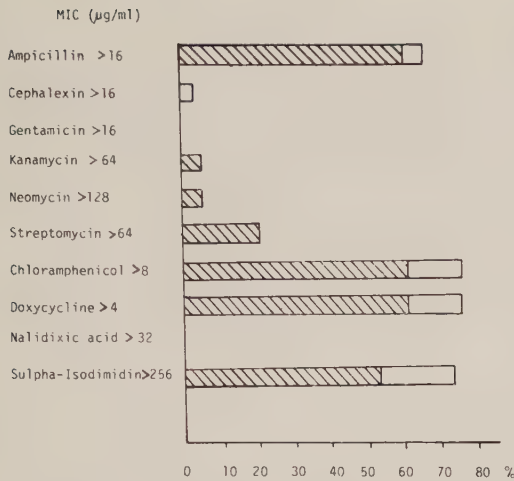


Fig. 3. Antibiotic resistance to 10 different antibiotics. Serotype 0111:B4 ▨. Other serotypes □.

RESULTS

Sensitivity Testing

Fig. 1 and Fig. 2 show the MIC values for mecillinam and TMP-SMZ. For mecillinam, only strains of 0111:B4 had a moderately increased MIC value in the interval of 0.8–3.2 µg/ml, for TMP-SMZ, however, there was no such obvious correlation of MIC to serotype. One of the multi-resistant strains of serotype 0111:B4 was re-isolated from a patient after a 5 days' course of TMP-SMZ. The MIC-value for TMP-SMZ had then increased from 1 µg/ml before treatment to 32 µg/ml after treatment. Fig. 3 shows the number of resistant

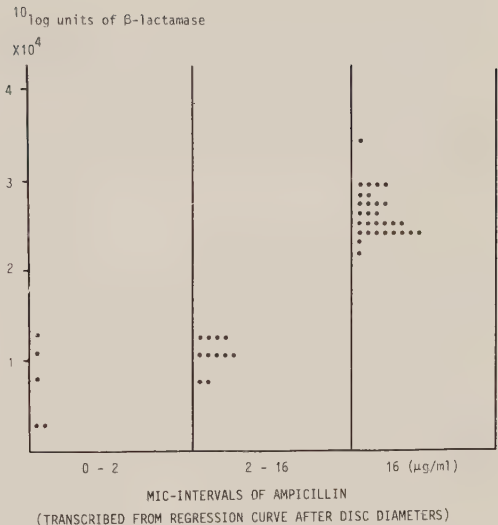


Fig. 5. Beta-lactamase activity related to susceptibility of ampicillin.

strains to 10 other antibiotics. Multi-resistant strains generally belong to serotype 0111:B4. Only 2 strains were resistant to kanamycin and neomycin, one to cephalexin. All strains were sensitive to gentamicin and nalidixic acid.

Beta-lactamase Determination

Fig. 4 and Fig. 5 show the production of beta-lactamase related to the susceptibility of mecillinam and ampicillin respectively. The 27 strains of serotype 0111:B4 with MIC-values for mecillinam

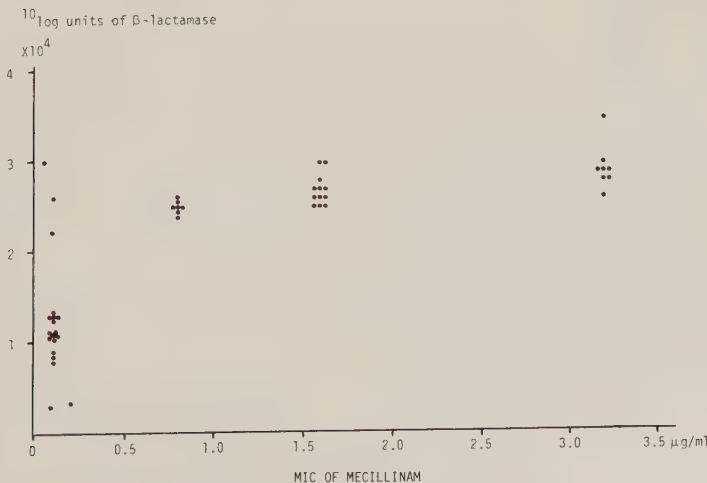


Fig. 4. Beta-lactamase activity related to susceptibility of mecillinam.

in the interval of 0.8–3.2 µg/ml all had increased beta-lactamase activity (Fig. 4). However, 3 strains with high beta-lactamase activity had low MIC-values for mecillinam (0.05–0.1 µg/ml). In contrast, Fig. 5 demonstrates that all strains with high beta-lactamase activity were resistant to ampicillin.

DISCUSSION

The treatment of widespread and potentially serious infections such as EPEC gastroenteritis poses some considerations concerning an «ideal» antibiotic: high efficacy against the offensive organism, low toxicity, low incidence of plasmid mediated R-factors and (especially in developing countries) low cost. Aminoglycosides and colistin have been widely used as effective drugs for *E. coli* infections although they may have some toxic effects. Regarding ampicillin, sulfonamides, tetracycline and chloramphenicol: widespread resistance of EPEC strains has been reported (1, 2, 4, 7, 10).

Mecillinam and TMP-SMZ are 2 relatively new drugs reported to have high efficacy against *E. coli* infections and low toxicity (3, 6). These 2 drugs were successfully used in the treatment of severe EPEC gastroenteritis (12) and *in vitro* investigation of susceptibility confirmed that the MIC-values for mecillinam were 5–10 times below the serum concentrations usually obtained with the given dosages of the drug (40 mg per kg of bodyweight i.m.) (11). A moderate increase of MIC values to mecillinam was always associated with ampicillin resistance and confined to the most frequent serotype (O111:B4). It is not clear whether increased MIC for mecillinam depends on increased beta-lactamase activity, since the few ampicillin resistant strains of other serotypes, with increased beta-lactamase activity as well, had low MIC values for mecillinam. The susceptibility of these isolates to TMP-SMZ was generally good. However, the marked increase of MIC in one of the strains after treatment with TMP-SMZ is an observation.

This investigation shows that clinical EPEC isolates from Ethiopia are frequently multi-resistant, many of the isolates belonging to serotype O111:B4. Modern drugs such as mecillinam and TMP-SMZ should be considered as alternatives beside established treatment like aminoglycosides.

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Running title:

Pathogenesis of enteropathogenic Escherichia coli.

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Pathogenesis of diarrhoea caused by enteropathogenic Escherichia coli: Studies of enterotoxin production, invasive ability and surface antigens.

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Abstract

The pathogenicity of enteropathogenic Escherichia coli (EPEC) is not yet clear. Faecal E. coli strains mainly from Ethiopian children with and without diarrhoea were investigated. Diarrhoeal strains included 78 Ethiopian and three Swedish EPEC strains. Twelve enterotoxigenic strains served as positive controls and 16 non-EPEC strains from healthy children as negative controls. The strains were assayed for heat-labile and heat-stable enterotoxins, invasive ability and the presence of colonization factor antigen (CFA). Studies on adhesive factors also included hemagglutination reactions and hydrophobic interaction chromatography. Attempts were made to demonstrate EPEC-specific adhesive surface antigen by immunizing rabbits with the adhesive EPEC strain H19/1 followed by adsorption of the antiserum with a non-adhesive plasmid cured derivative (H19/23). The EPEC strains were non-invasive, non-toxigenic (save two) and lacked CFA (save one). Hemagglutination indicated frequent presence of type 1 pili in EPEC and non-EPEC strains. No hydrophobic interaction or EPEC specific surface antigens were demonstrated. EPEC strains obviously do not possess the virulence factors of enterotoxigenic and invasive strains and other test models might provide more information.

The virulence of enteropathogenic Escherichia coli (EPEC) has been demonstrated by epidemiological observations (1,2,3,4) and experiments on adult volunteers (5,6). Subsequently, it has been shown that generally EPEC strains of classical serogroups do not produce the heat-labile or heat-stable enterotoxins of enterotoxigenic Escherichia coli strains (ETEC) and are non-invasive (7,8). However, some reports suggest EPEC-specific enterotoxins (9,10,11).

The ability of microorganisms to stick to target epithelium is now considered an essential step in most infections (12). Evans et al have demonstrated plasmid-controlled colonization factor antigen (CFA) in ETEC strains. Rabbits were immunized with the adhesive strain H10407 that possessed the plasmid-controlled CFA_I-antigen. A derivative strain that had lost its plasmids (H10407-P) was used to adsorb the antiserum and yield a specific CFA_I antiserum (13). Evans et al also demonstrated that the presence of CFA_I was associated with mannose-resistant hemagglutination (MRHA) of human red blood cells (14). By similar methods a second colonization factor (CFA_{II}) causing MRHA of bovine erythrocytes was detected in other ETEC strains (15).

Studies by Duguid and Brinton (16,17) on adhesins of gramnegative bacteria had previously shown an association of bacterial pili-structures, visualized by electron microscopy, and hemagglutination of erythrocytes from different animal species. An example is Type 1 or common type pili that causes mannose-sensitive hemagglutination (MSHA) of erythrocytes from guinea-pig and chicken (16). Accordingly, hemagglutination technique using erythrocytes from different species has been a screening tool in order to search for adhesive properties of bacteria (18,19).

The CFA adhesins have hydrophobic properties which can be demonstrated by so called Hydrophobic Interaction Chromatography (HIC). Bacteria possessing the hydrophobic adhesins bind to Phenyl Sepharose in the presence of buffered ammonium sulfate (20,21). EPEC strains have not been tested in this assay.

EPEC strains generally lack the ETEC-associated CFA-adhesins (22).

Williams et al 1978 demonstrated in vitro adherence of the EPEC strain H19/1 (026:H11) to fetal intestinal mucosa of human origin (23).

The adhesion was associated with colicine production and multi-resistance to different antibiotics. All these properties were shown to be caused by transmissible plasmids. Removal of the plasmids using a so called plasmid curing agent (ethidium-bromide) resulted in a non-adhesive strain (H19/23). Moreover, genetic transfer of plasmids to a recipient non-adhesive E. coli (E. coli K12, resistant to nalidixic acid) gave an adhesive recipient strain.

The aim of this study was to investigate EPEC strains isolated from cases of infantile diarrhoea in Ethiopia and Sweden (24,25,26) for the presence of enterotoxins, invasive ability and adhesive properties indicated by hemagglutination properties and hydrophobic interaction chromatography. The aim was also to search for an EPEC specific surface antigen in strain H19/1 employing the methods described by Evans et al (13).

Material and methods

Bacteria. EPEC and ETEC strains were collected in Ethiopia (24,25) and Sweden (26) 1977 through 1979. Non-toxigenic E. coli strains of non-EPEC serogroups were also isolated from healthy Ethiopian

children. Fresh isolates were preserved in Trypticase soy broth containing 15% WT/vol glycerol, stored at -70°C . The EPEC strains H19/1 (026:K60:H11) and the plasmid-cured derivative H19/23 were kindly supplied by P H Williams and A S McNeish, University of Leicester, England. The ETEC strain H10407 (078:K80:H11) and the plasmid-cured derivative H10407-P were kindly supplied by D G Evans, Houston, Texas. Strain G1253 (0147:K⁻,K88ac:H19) was supplied by F and I Ørskov, Statens Seruminstitut, Copenhagen, Denmark and served as positive control for HIC studies. An invasive E. coli strain (0124) served as positive control in studies of invasive ability. This strain and the lactose-negative nalidixic acid resistant E. coli K12 were kindly supplied by R Möllby, the National Bacteriological Laboratory (NBL), Stockholm.

Culture Media and Growth Conditions. For optimal production of colonization factor antigens cultures were performed overnight on so called CFA-agar at 37°C (14). For most experiments, bacterial suspensions were prepared from the CFA-agar plate in phosphate buffered saline (PBS). For optimal expression of type 1 pili, the bacteria were grown in a static trypticase soy broth culture for 48 hours at 37°C (16).

Invasive Ability. The assay was performed according to Pedersen et al using a HeLa cell culture (27).

Enterotoxin Assays. Assay for heat-labile enterotoxin (LT) was performed by R Möllby, NBL, Stockholm according to the Y1 adrenal cell test (28). Six selected strains (one ETEC, five EPEC) were tested in the Vero cell assay for vero cell cytotoxin (VT) by J Konowalchuk,

Ottawa, Canada (9). The presence of heat-stable enterotoxin ST was assayed by the suckling mouse test (29).

Hemagglutination (HA). Suspensions of bacteria in PBS (10^{10} bacteria/ml), cultured as described above, were mixed with an equal volume of 1% erythrocytes (washed three times) in PBS. Erythrocytes from different species were used: human, bovine, chicken and guinea-pig. The HA studies were performed on glass depression slides and recorded after two minutes gentle agitation at 20°C and also at 4°C . The tests were performed in the absence and presence of 1% D-mannose in PBS (18,21). HA studies and studies on hydrophobic interaction chromatography (described below) were performed in cooperation with Torkel Wadström and Ahmed Faris at the Biomedical Center, Uppsala, Sweden.

Hydrophobic Interaction Chromatography (HIC). Bacteria grown on CFA-agar as described above and also in Tryptone yeast broth overnight at 37°C (centrifuged and washed once) were suspended in 1M ammonium sulfate buffered with 10mM sodium phosphate buffer (pH 6.8) to a density of $2-4 \times 10^{10}$ bacteria/ml. Pasteur pipettes were packed with Phenyl sepharose gel (Pharmacia AB, Uppsala, Sweden) and the gel was equilibrated with the suspension fluid. One hundred μl of bacterial suspension was applied on top at the column and then eluted with the suspension fluid. The E. coli strain G1253 served a HIC-positive control. A positive interaction test between the bacteria and the gel is demonstrated by a visually clear eluate from the pipette (21).

Immunological Studies on Surface Antigens. Antisera prepared against CFA_I and CFA_{II} were supplied by Torkel Wadström (13,15). The bacteria

were investigated for the presence of the ETEC-associated antigens by slide agglutination.

The previously described strain H19/1 with plasmid-mediated ability to adhere to human fetal intestinal mucosa was investigated for the presence of surface antigens according to Evans et al (13). Five rabbits were immunized by i.v. injections of a deepfrozen badge of living bacteria suspended in normal saline. During the first four weeks injections were given three times weekly, week 5-8 twice weekly. The doses started with 0.5×10^6 bacteria increasing to 10^8 bacteria week 8 after which the rabbits were bled. Titer response was controlled using a suspension of strain H19/1 boiled for one hour as antigen (Widal technique). Antiserum from two rabbits with the highest titer response ($<1/4 - 1/1024$) was adsorbed with strain H19/23 according to Evans et al until no reaction with strain H19/23 was observed (13). Adsorbed antiserum was tested against strain H19/1 by tube agglutination. Bacteria grown on CFA agar were suspended in normal saline (10^9 bact/ml) added with 1 mg sodium azide/ml to prevent further growth (30). The antigen suspension was mixed with two-fold dilutions of antiserum and incubated overnight 37°C . A similar assay was also performed using bacteria boiled one hour and incubated at 50°C .

Genetic Experiments. An Ethiopian EPEC strain EtE1 (0111:B4) carrying multiple antibiotic resistance was selected as a donor strain for transferring antibiotic resistance to a recipient strain E. coli K12. The plasmid transfer was performed according to Williams et al (23). Rabbit antiserum against EtE1 (prepared as described) was used in tube agglutination (as described) against different derivatives of E. coli K12 which had acquired antibiotic resistance from EtE1.

Results

Laboratory results of EPEC, ETEC and control strains are summarized in Table 1.

Enterotoxin Assays. Only two Ethiopian EPEC strains (serogroup 0114) were LT positive, none was ST or VT positive.

Invasive Ability. Forty-five EPEC strains (tested within two months after isolation) were all negative according to the HeLa cell test.

Hemagglutination (HA) Reactions and Agglutination with CFA_I and CFA_{II} Antiserum. Mannose resistant HA (MRHA) of human erythrocytes was present and correlated completely with the agglutination of CFA_I antiserum in 10/11 of Ethiopian ETEC 078 strains. MRHA of bovine erythrocytes was observed in 7/11 ETEC strains but no strain was agglutinated by CFA_{II} antiserum.

Among 78 Ethiopian EPEC strains only one showed MRHA (to human and bovine erythrocytes). This strain of serogroup 044 was nontoxigenic and CFA_I positive. No other EPEC strain was agglutinated by CFA_I or CFA_{II} antiserum.

After 48 hours growth in static broth 69/78 Ethiopian EPEC strains expressed mannose-sensitive HA (MSHA) of chicken and guinea-pig erythrocytes. This was also observed with 12/16 *E. coli* control strains from healthy Ethiopian children. Two of the latter strains expressed MRHA to human erythrocytes without the presence of CFA.

Hydrophobic Interaction Chromatography (HIC). Weakly positive HIC

Table 1. Enterotoxigenicity, invasive ability, hemagglutination properties, presence of CFA and HIC reaction in Ethiopian and Swedish E. coli strains of infantile diarrhoea. NT = not tested. (Results are expressed as number of positive strains related to number of tested strains).

Strains	LT+	ST+	VT+	Invasive+	MRHA ⁺ _{human}	MRHA ⁺ _{bovine}	MSHA ⁺ _{chicken}	MSHA ⁺ _{guineapig}	CFA _I	CFA _{II}	HIC+
Ethiopian EPEC 1)	2/78	0/78	0/5	0/41	1/78	1/78	69/78	69/78	1/78	0/78	*0/40
Swedish EPEC 2)	0/3	0/3	NT	0/3	0/3	0/3	2/3	2/3	0/3	0/3	NT
Swedish <u>E.coli</u> 078	0/1	0/1	NT	0/1	0/1	0/1	1/1	1/1	0/1	0/1	NT
Ethiopian ETEC 078	9/12	10/12	0/1	NT	10/11	7/11	11/11	11/11	10/11	0/11	2(+)/8
Ethiopian <u>E.coli</u> control strains	0/16	0/16	NT	NT	2/16	0/16	12/16	12/16	0/16	0/16	NT

1) 0-Serogroup: 044(8), 055 (10), 086 (1), 0111 (39), 0114 (4), 0119 (4), 0126 (5), 0127 (6), 0128 (1).

2) 0-Serogroup: 086 (1), 0126 (1), 0127 (1).

reaction was observed in 2/8 Ethiopian ETEC strains but all tested EPEC strains were negative. The control strain G1253 was strongly positive.

Immunological Studies. Antiserum against strain H19/1 showed equal agglutination in titer 1/1280 with strain H19/1 and its plasmid cured derivate H19/23. After adsorption of the antiserum with strain H19/23 no agglutination was observed to either strain.

Genetic Transfer Studies. Five recipients of E. coli K12 which had acquired multiple antibiotic resistance from the donor strain EtE1 (Ethiopian EPEC 0111:B4) were not agglutinated by antiserum against the donor strain. This implicates that no plasmids coding for surface antigens were transferred together with the R-factor.

Discussion

The ETEC associated enterotoxins LT and ST are rarely found in EPEC strains. In the present study only two strains (serogroup 0114) expressed LT accounting for 2% of all strains. Cytotoxins demonstrated by the Vero cell assay do not provide a general explanation of EPEC virulence. Reports of EPEC specific enterotoxins only concern a very limited number of strains (10,11).

EPEC strains are non-invasive - this finding was confirmed in 45 fresh isolates from Ethiopia and Sweden.

EPEC strains do not express the ETEC associated surface antigen (CFA) mediating specific receptor binding to intestinal mucosa cells.

Hemagglutination studies commonly demonstrate typ 1 pili in EPEC strains but these are also found in many non-pathogenic E. coli

strains. Otherwise HA does not provide a mean of discriminating EPEC.

Reports of EPEC specific adhesive factors are few (22,23). Attempts to demonstrate a surface antigen associated with an EPEC specific colonization factor proved not successful in this study. Other enteropathogenic mechanisms similar to salmonellosis have been investigated in a rabbit model (31,32). Clinical and morphological studies of some human EPEC infections resembling the rabbit model have lately been reported (33,34,35). Further studies are needed to elucidate the virulence of EPEC strains.

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